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# HEAT TREATMENTS FOR THE CURE OF YELLOWS AND OTHER VIRUS DISEASES OF PEACH[1]

L. O. KUNKEL

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## INTRODUCTION

Curing peach trees of the yellows disease by incubating them at approximately 35° C. for two weeks or longer was reported in 1935 (10). Further studies on the effectiveness of moderately high temperatures for the cure of yellows have now been completed. Since the virus of this disease is not transmitted mechanically, except by grafting, its resistance to high temperatures can not be studied by means of techniques usually employed in work on viruses transmitted by ordinary mechanical inoculations. The fact that yellows virus can be inactivated by exposure of trees to temperatures that cause little or no injury to peach tissues makes an *in vivo* study of its thermal relationships possible. Since most plant viruses are not destroyed by heat treatments sublethal for their hosts, it seemed worthwhile to determine with some accuracy the sensitiveness of yellows virus to heat inactivation at several different temperatures. It also seemed desirable to study the effects of heat treatments on other virus diseases of peach at this time, since experiments including tests of other diseases frequently could be carried out almost as easily as experiments with yellows alone. The results obtained are reported in this paper.

## MATERIALS AND METHODS

The trees used in heat-treatment studies were grown under conditions similar to those described in an earlier paper (11) from seed purchased of a dealer at Germantown, Pennsylvania. All seedlings were selected for uniformity in size, vigor, and type of growth, and were less than 2 years old when taken for experiment. The yellows virus used was the same as that mentioned in the paper to which reference has already been made.

Three different methods were employed in studying the effects of high temperature on diseased peach tissues. The first method involved the incu-

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bation of actively growing diseased trees in a thermostatically controlled room lighted by means of a window. The trees were placed on a table directly in front of the window. The room was held at a temperature varying from about 34.4° C. to 36.3° C. After incubation in the room for varying periods of time, the trees were removed to a greenhouse and kept under observation. When they had produced normal-appearing foliage and a healthy type of growth over a period of a year or more, they were considered to have been cured. Subinoculations from such trees invariably failed to cause disease. This method of treatment was satisfactory in many respects, and was used in the cure of about 300 trees. The chief disadvantage of the method was that it required long periods of incubation. Attempts to shorten the periods by raising the temperature proved impracticable, because of the burning of foliage and tender young branches. Another disadvantage of the method was that the virus was not inactivated at a uniform rate in different parts of the trees. It was destroyed in from 4 to 6 days in slender young branches, in about 2 weeks in thick stems and branches, and in from 2 to 4 weeks in large roots. Trees with virus persisting only in their roots had the appearance of being cured and produced healthy-looking foliage for several months following treatment, but symptoms of disease would then appear in shoots produced near the soil level and the virus would spread upward into the tops. This lack of uniformity in rate of destruction of virus in roots and tops is believed to have been due chiefly to cooling caused by evaporation of water from the moist soil in which the roots were imbedded.

In order to avoid the difficulty encountered in curing roots, infected trees were sometimes treated before virus had had time to spread from the point of inoculation into the roots. In these instances the trees were always treated while the disease was in an early stage of incubation, *i.e.*, within from 3 to 10 days after inoculation. This modification of the method gave fairly uniform results and eliminated the difficulty of having virus persist in roots after a treatment that had cured the tops, but it had the disadvantage of requiring application of treatment before the appearance of symptoms.

The second method consisted in the immersion of dormant trees for a short period of time in a tank of water held at about 50° C. The trees were removed from the pots in which they had been grown and the earth washed from their roots. After treatment they were repotted in fresh soil. This method was highly effective for the cure of yellows, but could be used only on trees in a dormant condition. While it was satisfactory for curing trees, it was not well adapted for studying virus inactivation. Even with this method of treatment yellows virus was more readily destroyed in slender than in thick stems and branches. The lack of uniformity in rate of destruction of virus in stems of different diameters was of little consequence when the object in view was to effect a cure, but it became important when tests

were undertaken to determine the effectiveness of treatments at any given temperature. Since it seemed desirable to eliminate, as far as possible, this source of error in studying the thermal relationships of the yellows virus, a third method of treating diseased tissues was adopted. It consisted in immersing pieces of yellows branches about 5 inches long and 3/32 of an inch in diameter in a thermostatically controlled water bath in which the water was kept in constant motion by means of an electrically propelled stirrer. All leaves were removed from the branches before treatment. After exposing the cuttings for varying periods of time, one or more buds were removed from each and inserted in a healthy young tree. If virus in the buds had been destroyed, the tree remained healthy; if not, it came down with yellows. When bud sticks of current season's growth were carefully selected for uniformity in thickness, hardness, and maturity, this method gave fairly consistent and reproducible results. It was employed in most of the experiments to be reported.

Rosette and little-peach viruses used in heat-treatment experiments were from the sources mentioned in another paper (11). Red-suture virus was obtained from Donald Cation, of East Lansing, Michigan. All of the 3 methods described above were used in treating the diseases caused by these viruses.

## EXPERIMENTAL

### Appearance of Cured Trees

When a tree having yellows was incubated in the hot room described above for a period of time sufficiently long to destroy all the virus in its tissues and was then placed in a greenhouse and held under favorable conditions, the growing buds produced only healthy-appearing leaves and stems. As is well known, the branches of yellows trees assume a more upright habit of growth than is normal for healthy trees. When the upright-growing lateral branches were cured, they at once began to produce less upright-growing tips and all side branches formed subsequent to the cure grew out in the spreading manner characteristic of healthy trees. Figure 1 shows a side branch from a tree cured of yellows by heat treatment. The lower portion of the branch was produced while the tree was diseased, the upper portion after it was cured. The location of the terminal bud at the time the tree was subjected to heat treatment is shown by the angle that marks the change in direction of growth of the branch. On being cured, the branch immediately changed its habit of growth, as well as the type of leaves and stem internodes it produced. Subinoculations from both the upper and lower portions of the branch failed to transmit disease. The effect of yellows on direction of growth is shown in a more striking manner by figure 2, which presents a portion of the main stem of a young tree with a side branch

FIG. 1. Lateral branch from peach tree cured of yellows. The lower portion of the branch was produced before and the upper portion after the tree was cured. The lower portion bears small narrow chlorotic leaves that are typical for yellows disease, while the upper portion bears large deep green leaves separated by internodes of normal length. The angle made by the two sections marks the location of the terminal bud at the time of cure.

attached. The tip of both stem and branch was removed in order to reduce the size of the specimen to be photographed. The lower part of the branch bears no leaves. This section was produced while the tree was healthy. The angle between this first section and the stem is normal for a healthy tree. The second or middle section of the branch bears small narrow leaves and shows the upright habit of growth characteristic of side branches affected by yellows. This section was produced after the tree became diseased. The

third section of the branch bears large green leaves. A straight line drawn through the long axis of this portion to the main stem would subtend an angle approximately equal to that subtended by the first section. The third section was produced after the tree was cured by heat treatment. The terminal bud of this branch produced normally-directed growth both before it became diseased and after it was cured. It produced abnormally-directed growth while diseased. Subinoculations made from each of the 3 sections shortly after the photograph was taken caused no infections and proved that the branch was virus-free throughout. The tissues formed while the branches shown in figures 1 and 2 were diseased did not lose the symptoms of yellows after the branches were cured. The small, narrow leaves and

FIG. 2. A portion of the main stem of a young peach tree with side branch attached. The lower section of the side branch was produced while the tree was healthy, the middle section while it was diseased, and the upper section after it was cured. The terminal bud of the branch produced normally-directed growth both before the tree became diseased and after it was cured. The bud produced abnormally-directed growth as well as small leaves and short internodes while the tree was diseased.

short internodes produced while virus was present retained the light yellow color that they had before treatment. The new growth from cured trees bore no symptoms of yellows. Only virus of the masked-symptom type could have been present. If the treatments merely attenuated yellows virus, one would expect that different exposures would attenuate it to different degrees, and that some attenuated strains would produce mild but visible symptoms of disease. No evidence of this was obtained. It is known that weak strains of tobacco mosaic protect against ordinary or severe strains of this disease (9), and likewise that little-peach disease protects against

yellows (11). If trees that have been cured of yellows by heat treatments, or trees that have been inoculated with buds from cured trees were affected by an attenuated yellows virus, they would be expected to show immunity from the type of yellows caused by unattenuated virus. Accordingly, cured trees and trees in which buds from cured trees had been inserted were inoculated with yellows buds. In all cases the trees came down promptly and showed typical symptoms of yellows. If any unattenuated virus had been present in trees cured of yellows, it should have multiplied, for, as has been proved experimentally, it does multiply in such trees when they are reinfected. Moreover, if unattenuated virus had been present, it should have been transmitted when subinoculations were made to healthy trees. As no evidence was obtained that virus either multiplied in, or could be transmitted from, cured trees, it is concluded that none was present, and that cure resulted from complete destruction of the causative agent.

## Heat Treatments for the Cure of Yellows

Since yellows trees were first cured by exposing them for long periods of time at approximately 35° C., a description will be given of a typical hot-room experiment. On October 1, 1934, 60 healthy seedling trees approximately 3½ feet tall, grown from seeds planted in pots during the preceding February, were selected for uniformity in size and vigor of growth. A yellows bud was inserted in the stem of each of 50 of the trees at a point about 2 feet above the soil level. The other 10 trees were not inoculated. About 1 month later all trees were taken from the greenhouse to a cold frame, in order to expose them to low temperatures that would induce dormancy. On December 20 they were returned to the greenhouse. By February 1, 1935, all of the trees had produced new growth, and all except 1 of those that were inoculated showed characteristic symptoms of yellows. On examination it was found that the bud used to inoculate the tree that failed to become infected had died. The buds used in inoculating the other 49 trees had lived. On February 7, 1935, 27 of the yellows trees were placed in the hot room described above. The other 22 diseased trees and the 10 healthy trees were left in the greenhouse to serve as controls. On each succeeding day, for a period of 27 days, one of the treated trees was returned to the greenhouse. All of the trees in this experiment were again transferred to a cold frame on September 11, 1935. On December 16 they were brought back into the greenhouse and forced into growth. The trees were held under observation for 14 months following treatment. Their appearance with respect to yellows symptoms after different intervals of time is shown in table 1. All trees kept in the hot room for 10 days or longer produced healthy-appearing new growth after the treatment. Those exposed for less than 10 days produced new growth bearing symptoms of yellows.

TABLE 1.—*Cure of peach yellows by incubation of trees in a hot room held at 34.4 to 36.3° C.*

| Period of exposure | Appearance of trees after | | | | | |
|---|---|---|---|---|---|---|
| | 54 days | 81 days | 105 days | 215 days | 365 days | 420 days |
| 1 day | D | D | D | D | D | D |
| 2 days | D | D | D | D | D | D |
| 3 " | D | D | D | D | D | D |
| 4 " | D | D | D | D | D | D |
| 5 " | D | D | D | D | D | D |
| 6 " | D | D | D | D | D | D |
| 7 " | D | D | D | D | D | D |
| 8 " | D | D | D | D | D | D |
| 9 " | D | D | D | D | D | D |
| 10 " | H | D | D | D | D | D |
| 11 " | H | D | D | D | D | D |
| 12 " | H | D– | D– | D | D | D |
| 13 " | H | D– | D– | D | D | D |
| 14 " | H | H | H | H | D | D |
| 15 " | H | D– | D– | D | D | D |
| 16 " | H | D– | D– | D | D | D |
| 17 " | H | H | H | D– | D | D |
| 18 " | H | H | H | D– | D | D |
| 19 " | H | H | H | H | H | H |
| 20 " | H | H | H | H | D | D |
| 21 " | H | H | H | H | D | D |
| 22 " | H | H | D– | D– | D | D |
| 23 " | H | H | D– | D– | D | D |
| 24 " | H | H | H | H | H | H |
| 25 " | H | H | H | H | H | H |
| 26 " | H | H | H | H | H | H |
| 27 " | H | H | H | H | H | H |

H = Healthy;
D = Diseased;
D– = Disease symptoms only in lower part of tree

After varying periods of time, as is indicated in table 1, yellows symptoms appeared in twigs produced on the stems of many previously healthy-looking treated trees. Such twigs always appeared first at or near the soil level. Subinoculations made from different parts of these trees proved that their healthy-appearing tops were free of virus but that their lower portions were infected. All healthy check trees remained healthy, and all diseased check trees diseased. Only 5 of the 27 treated trees were permanently cured. These 5 trees were exposed for periods of 19, 24, 25, 26, and 27 days, respectively. No explanation can be given as to why the tree exposed for 19 days was cured, while trees exposed for 20, 21, 22, and 23 days, respectively, were

not cured. Similar irregularities were observed in other heat-treatment experiments. The question might be raised as to whether or not the 5 trees that were still healthy in appearance 14 months after they were treated might not eventually come down with yellows. In the writer's experience, all treated trees that have remained free of symptoms after a vegetative period as long as 8 months are permanently cured. Eleven trees that were heat-treated in an earlier experiment showed no signs of disease 26 months after they were removed from the hot room. Subinoculations made from both stems and roots of 2 of the trees approximately 1 year after treatment failed to transmit yellows. The experiments prove that yellows trees can be cured by prolonged exposure to air held at about 35° C.

Trees as old as those used in the experiment described above were not seriously injured by hot-room exposures lasting as long as 40 days, but younger trees were unable to survive such prolonged treatments. As was stated in the description of methods, trees are more readily cured when treated before virus has had time to spread into their roots than when treated after roots are infected. Tender young trees can be cured of yellows very readily if they are treated shortly after infection.

In order to present data illustrating these facts, another heat-treatment experiment that was started at the same time as that just reported will be described. Forty-eight healthy seedling trees about 6 months old and approximately 18 inches high were selected for uniformity in size and vigor. Each of 45 of the trees was inoculated by inserting a yellows bud in the stem at a point 6 to 8 inches above the soil level. The other 3 trees were not inoculated and served as controls. Three days later 42 of the 45 inoculated trees were placed in the hot room. The other 3 inoculated trees were left in the greenhouse to serve as controls. At daily intervals over a period of 2 weeks, 3 trees were transferred from the hot room to the greenhouse. Shortly after the treatments were completed, all trees except those killed by heat were placed in a cold frame to promote dormancy. They were taken back to the greenhouse during the last week in December and were held under observation until October 1, 1935, when the experiment was ended. All of the trees exposed to treatment for 6 days or longer, and 1 of the trees exposed for only 5 days, died. The buds used in inoculating lived in all trees that survived treatment. The trees that were treated for 1, 2, and 3 days came down with yellows, as did the 3 inoculated untreated control trees. The 3 that were treated for 4 days and the 2 that survived 5 days of treatment remained healthy. The 3 noninoculated control trees likewise remained healthy. A tree from each of the sets that survived treatment is pictured in figure 3. The photograph was taken February 16, 1935, and shows the trees as they appeared 4½ months after treatment. The 3 diseased trees standing on the left in the picture were exposed for 1, 2, and 3

days, respectively, while the 2 healthy trees standing on the right were exposed for 4 and 5 days, respectively. Under the conditions of this experiment, the yellows virus was destroyed by treatments lasting 4 and 5 days, but not by treatments lasting 3 days or less. The experiment proves that yellows virus in newly-infected young trees is destroyed by hot-room treatments lasting about 4 days.

It was thought that successive treatments involving 1- or 2-day exposures in the hot room might prove effective for the cure of yellows and cause less injury to trees than prolonged continuous exposures. The following experiment was made for the purpose of testing this possibility. Twelve trees that had been affected by yellows for about 2 months were divided into 4 sets of 3 trees each. On April 30, 1935, 3 of the sets were placed in the hot room. The 4th was not treated and served as a control. The 1st set was returned to the greenhouse after continuous incubation in the hot room for 30 days. The 2nd set was treated by incubating the trees in the hot room on alternate days during a period of 60 days. The 3rd set was similarly treated during alternate 2-day periods. The trees were held in a greenhouse on the days they were not in the hot room. The total length of time each set of treated trees was kept in the hot room amounted to 30 days, but the 2nd set was exposed during 30 different 1-day periods, while the 3rd set was exposed during 15 different 2-day periods. All trees were held under observation for a year following treatments. Those that were kept in the hot room continuously for 30 days were cured, but none that were subjected to intermittent treatments were cured or even favorably affected insofar as could be judged by symptoms. However, they suffered no injury from the treatments. The effectiveness of successive treatments lasting longer than 2 days was not investigated.

An experiment designed to determine the period of treatment necessary for the curing of dormant trees by the second method will now be described. The experiment was planned after preliminary tests had shown the approximate time required to inactivate virus at 50° C. Eight of a group of 10 healthy young trees about 11 months old were inoculated with yellows virus by inserting a yellows bud in the stem of each tree at a point about 2 feet above the soil level on September 3, 1934. All of the inoculated trees showed symptoms of yellows within 6 weeks. The 2 noninoculated control trees remained healthy. During the first week in November the trees were transferred from the greenhouse, in which they had been grown, to a cold frame, where they were allowed to become dormant. On February 12, 1935, they were returned to the greenhouse. Soil was removed from their roots 2 days later, after it had had time to thaw. Six of the trees were then treated by immersing them for periods of time varying by 2-minute intervals from 2 to 12 minutes in a tank of water held at approximately 50° C. The other 2

FIG. 3. Five trees used in a hot-room experiment. All of the trees were infected with yellows virus before treatment. The 3 trees shown on the left were treated for 1, 2, and 3 days, respectively; the 2 trees on the right for 4 and 5 days, respectively. The picture shows that the latter were cured and that the former were not cured.

TABLE 2.—*Thermal inactivation points of peach-yellows virus in bud sticks*

| 34.35° C. | | | 38° C. | | 40° C. | | 42° C. | | | | 44° C. | | 46° C. | | 48° C. | | 50° C. | | | | | | | 52° C. | | 54° C. | | 56° C. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time | Effect | Effect | Time | Effect | Time in minutes | Effect | Time in minutes | Effect | Time in minutes | Effect | Time in minutes | Effect | Time in minutes | Effect | Time in minutes | Effect | Time in minutes | Effect | Time in minutes | Effect | Effect | Time in minutes | Effect | Time in minutes | Effect | Time in minutes | Effect | Time in minutes | Effect |
| 1 hr. | + | + | 3 hrs. | + | 20 | + | 25 | + | 18 | + | 5 | + | 5 | + | 2 | + | 2 | + | 1 | + | + | 1 | + | ½ | + | ½ | 0 | ¼ | 0 |
| 5 hrs. | + | + | 4 " | + | 30 | + | 30 | + | 21 | + | 10 | + | 10 | + | 4 | + | 4 | 0 | 2 | + | + | 2 | + | 1 | 0 | 1 | + | ½ | 0 |
| 10 " | + | + | 5 " | + | 40 | + | 35 | + | 24 | + | 15 | + | 15 | 0 | 6 | + | 6 | 0 | 3 | + | 0 | 3 | 0 | 1½ | 0 | 1½ | 0 | ¾ | 0 |
| 20 " | + | + | 6 " | + | 50 | + | 40 | 0 | 27 | + | 20 | + | 20 | 0 | 8 | + | 8 | 0 | 4 | 0 | 0 | 4 | 0 | 2 | 0 | 2 | 0 | 1 | 0 |
| 30 " | + | + | 7 " | + | 60 | + | 45 | 0 | 30 | + | 25 | + | | | 10 | + | 10 | 0 | 5 | 0 | 0 | 5 | 0 | 2½ | 0 | 2½ | 0 | 1¼ | 0 |
| 2 days | + | + | 8 " | + | 70 | + | 50 | 0 | 33 | + | | | | | 12 | + | 12 | 0 | 6 | 0 | 0 | 6 | 0 | 3 | 0 | 3 | 0 | 1½ | 0 |
| 3 " | + | + | 9 " | + | 80 | + | 55 | 0 | 36 | + | | | | | 14 | 0 | 14 | 0 | 8 | 0 | 0 | 7 | 0 | 3½ | 0 | 3½ | 0 | 1¾ | 0 |
| 4 " | 0 | + | 10 " | + | 90 | + | 60 | 0 | 39 | + | | | | | | | 16 | 0 | 10 | 0 | 0 | 8 | 0 | | | | | 2 | 0 |
| 5 " | 0 | 0 | 11 " | 0 | 100 | + | 65 | 0 | 42 | 0 | | | | | | | 18 | 0 | 12 | 0 | 0 | 9 | 0 | | | | | | |
| 6 " | 0 | 0 | 12 " | 0 | 110 | + | 70 | 0 | 45 | + | | | | | | | 20 | 0 | 14 | 0 | 0 | 10 | 0 | | | | | | |
| 7 " | 0 | 0 | 13 " | 0 | 120 | + | 75 | 0 | 48 | 0 | | | | | | | 22 | 0 | 16 | 0 | 0 | | | | | | | | |

+ indicates that bud transmitted disease;
0 indicates that bud did not transmit disease.

yellows trees and the 2 healthy control trees were not treated. Immediately after treatment, all trees were repotted and placed in a greenhouse. A month later the tree held in the bath for only 2 minutes showed yellows symptoms to the same extent as the untreated yellows trees; but the other 5 treated trees appeared to be healthy. Two and one-half months after treatment, the 3 trees held in the bath for 4, 6, and 8 minutes, respectively, showed symptoms of yellows. The tree exposed for 4 minutes was thoroughly diseased, but the tree exposed for 6 minutes showed yellows symptoms only in shoots from the stem and large branches, and the tree exposed for 8 minutes only in shoots from the main stem. The 2 trees exposed for 10 and 12 minutes, respectively, were held under observation for a period of 13 months following the treatment. These trees grew vigorously and never showed symptoms of yellows. The experiment proves that, under the conditions described, dormant trees may be cured by treatments lasting 10 minutes or longer.

The third method of treatment described above was employed in experiments designed to determine the time required for thermal inactivation of virus at a number of different temperatures. Table 2 presents a summary of the results obtained in some of these experiments. The temperatures employed varied from 34.35° C. to 56° C. The low temperature was approximately the same as the minimum temperature of the hot room in which trees treated by the first method were exposed. At such a low temperature an exposure of from 4 to 5 days was necessary to inactivate virus in the buds of treated sticks. It was inactivated by a 4-day exposure in the first, but not in the second experiment. The reason for this variability is not definitely known, but as temperature was accurately controlled, it is presumed to have been due to differences inherent in the bud sticks. Similar irregularities appear in the data from some of the other experiments. At 38° C. virus was inactivated in 11 hours, but not in 10 hours or in shorter periods of time. The exposure periods employed with bud sticks held at 40° C. and at 44° C. were not sufficiently long to inactivate virus. These tests show that 2 hours at 40° C. and 25 minutes at 44° C. were insufficient for inactivation. Some other experiments carried out at the same temperatures, but under conditions that gave less accurate control than was had in the experiments reported in table 2, indicate that only slightly longer treatments would have given inactivation at 40° C. and 44° C.

The effectiveness of other temperatures for the destruction of virus is shown by the results of the other experiments summarized in the table. A 40-minute treatment was sufficient at 42° C., a 15-minute treatment at 46° C., a 14-minute treatment at 48° C., a 1-minute treatment at 52° C., and a 1½-minute treatment at 54° C. The table shows that at 54° C. virus was destroyed by a ½-minute treatment but not by a 1-minute treatment. A

somewhat similar inconsistency appears in the data from one of the experiments in tests at 42° C. At 56° C. virus was destroyed by treatment for ¼ minute, which was the shortest period tested. The results of 4 experiments on inactivation time at 50° C. are reported in the table. Virus was destroyed by a 4-minute exposure but not by a 2-minute exposure in each of these experiments. It was destroyed by a 3-minute treatment in 2 experiments but not in a third.

The shortest inactivation periods obtained at several different temperatures are indicated by points used in plotting the curve presented in figure 4, in which a logarithmic scale was employed to show time in hours on the abscissa and an arithmetical scale to show temperature in degrees centigrade on the ordinate. The curve is steeper at high than at low temperatures. This indicates that inactivation of yellows virus in living bud tissues is not governed by a linear function. The minimum temperature at which yellows buds can be cured has not yet been determined.

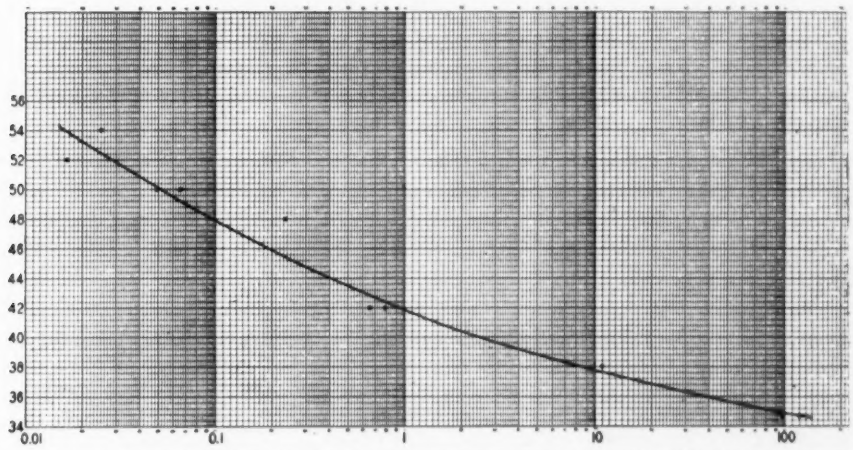


FIG. 4. Curve showing the relation of temperature and time of treatment to thermal inactivation of yellows virus in bud sticks. A logarithmic scale is employed to show time in hours on the abscissa and an arithmetical scale to show temperature in degrees centigrade on the ordinate.

The thermal inactivation time for yellows virus at the different temperatures investigated was found to be much shorter than the time required to kill bud tissues. In fact, the exposure periods that bud tissues were able to endure at high temperatures were so much greater than the thermal inactivation time of virus that no difficulty was experienced in curing buds without injuring them. The interval between the minimum inactivation period of the virus and the maximum endurance period of buds was greater at low than at high temperatures. Two experiments carried out at approxi-

TABLE 3.—*Inactivation time of yellows virus and endurance time of diseased buds at 45° and 50° C.*

| Treatment at approximately 45° C. | | | | | | Treatment at approximately 50° C. | | |
|---|---|---|---|---|---|---|---|---|
| Exposure time | Effect on virus | Effect on bud | Exposure time | Effect on virus | Effect on bud | Exposure time | Effect on virus | Effect on bud |
| 5 mins. | + | bud alive | 170 mins. | 0 | bud alive | 2 mins. | + | bud alive |
| 10 " | + | " " | 180 " | 0 | " " | 4 " | 0 | " " |
| 15 " | + | " " | 190 " | 0 | " dead | 6 " | 0 | " " |
| 20 " | + | " " | 200 " | 0 | " alive | 8 " | 0 | " " |
| 25 " | 0 | " " | 210 " | 0 | " " | 10 " | 0 | " " |
| 30 " | 0 | " " | 220 " | 0 | " " | 12 " | 0 | " " |
| 35 " | 0 | " " | 230 " | 0 | " " | 14 " | 0 | " " |
| 40 " | 0 | " " | 240 " | 0 | " " | 16 " | 0 | " " |
| 45 " | 0 | " " | 250 " | 0 | " " | 18 " | 0 | " " |
| 50 " | 0 | " " | 260 " | 0 | " " | 20 " | 0 | " " |
| 55 " | 0 | " " | 270 " | 0 | " " | 22 " | 0 | " " |
| 60 " | 0 | " " | 280 " | 0 | " " | 24 " | 0 | " " |
| 65 " | 0 | " " | 290 " | 0 | " " | 26 " | 0 | " dead |
| 70 " | 0 | " " | 300 " | 0 | " dead | 28 " | 0 | " alive |
| 75 " | 0 | " " | 310 " | 0 | " alive | 30 " | 0 | " dead |
| 80 " | 0 | " " | 320 " | 0 | " " | 32 " | 0 | " " |
| 85 " | 0 | " " | 330 " | 0 | " " | 34 " | 0 | " " |
| 90 " | 0 | " " | 340 " | 0 | " dead | 36 " | 0 | " " |
| 95 " | 0 | " " | 350 " | 0 | " alive | 38 " | 0 | " " |
| 100 " | 0 | " " | 360 " | 0 | " dead | 40 " | 0 | " " |
| 105 " | 0 | " " | 370 " | 0 | " alive | 42 " | 0 | " " |
| 110 " | 0 | " " | 380 " | 0 | " " | 44 " | 0 | " " |
| 115 " | 0 | " " | 390 " | 0 | " dead | 46 " | 0 | " " |
| 120 " | 0 | " " | 400 " | 0 | " alive | 48 " | 0 | " " |
| 125 " | 0 | " " | 410 " | 0 | " " | 50 " | 0 | " " |
| 130 " | 0 | " " | 420 " | 0 | " " | 52 " | 0 | " " |
| 135 " | 0 | " " | 430 " | 0 | " " | 54 " | 0 | " " |
| 140 " | 0 | " " | 440 " | 0 | " dead | 56 " | 0 | " " |
| 145 " | 0 | " " | 450 " | 0 | " " | 58 " | 0 | " " |
| 150 " | 0 | " " | 460 " | 0 | " " | 60 " | 0 | " " |
| 155 " | 0 | " " | 470 " | 0 | " " | 62 " | 0 | " " |
| 160 " | 0 | " " | 480 " | 0 | " " | 64 " | 0 | " " |
| 165 " | 0 | " " | 490 " | 0 | " " | 66 " | 0 | " " |

+ indicates that bud transmitted disease.
0 indicates that bud did not transmit disease.

mately 45° C. and 50° C., respectively, will be described in order to show the effect of temperature on the length of this interval. The accurately controlled water bath used in the tests reported in table 2 was not available at the time these experiments were made. The heat treatments were carried out in a jar of water held in an electric oven. The temperature at which the water was maintained varied somewhat during both experiments. However, it did not go more than ½° C. above or below the desired temperature at any time. The results obtained are shown in table 3. Yellows virus was destroyed by a 25-minute treatment at 45° C. and by a 4-minute treatment at 50° C. All buds treated for 3 hours or less at 45° C. survived and grew on the trees into which they were transplanted. At this temperature buds were not invariably killed by exposure periods of less than about 7 hours. At 50° C. all buds treated for periods of 24 minutes or less survived, and all treated for 30 minutes or longer died. Somewhat erratic results with respect to the length of time that buds were able to survive treatment are shown by both experiments. The buds exposed at 45° C. for periods of 190, 300, 340, 360, and 390 minutes, respectively, died, while all of the other buds held at the same temperature for a period of 430 minutes or less survived. Similarly, the bud held at 50° C. for 28 minutes survived, while that held at the same temperature for 26 minutes died. No explanation can be offered for this variability in survival time of buds. The experiments show clearly that diseased peach tissues are able to endure much longer treatments than are necessary for the destruction of yellows virus. No evidence was obtained that tissues surviving treatment were injured or changed in any way, except for the changes associated with cure of yellows. Some of the cured buds that survived treatment in each experiment were forced into growth by cutting back the trees into which they were transplanted. The shoots produced were normal in every respect.

### HEAT TREATMENTS FOR THE CURE OF ROSETTE, LITTLE PEACH, AND RED SUTURE

The fact that peach trees affected by rosette may be cured by exposure to high temperatures has already been reported (10). In the course of work on treatment of trees for yellows, a number of experiments designed to test the possibility of curing little-peach and red-suture diseases were made. Both diseases yielded to treatments that proved effective in the cure of yellows. The results obtained with little peach in experiments employing the third method are summarized in table 4. Little-peach virus was inactivated by exposing buds for 115 minutes at 40° C., for 20 minutes at 46° C., for 8 minutes at 48° C., and for 3 minutes at 50° C. Somewhat erratic results were obtained with sticks exposed at 40° C. and at 42° C. The bud held at 40° C. for 105 minutes failed to transmit little peach, while that exposed at the same temperature for 110 minutes transmitted the dis-

TABLE 4.—*Thermal inactivation points of litte-peach virus in bud sticks*

| 40° C. | | 42° C. | | 44° C. | | 46° C. | | 48° C. | | 50° C. | | | 52° C. | | 54° C. | | 56° C. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time in minutes | Effect | Time in minutes | Effect | Time in minutes | Effect | Time in minutes | Effect | Time in minutes | Effect | Time in minutes | Effect | Effect | Time in minutes | Effect | Time in minutes | Effect | Time in minutes | Effect |
| 70 | + | 15 | + | 5 | + | 5 | + | 2 | + | 1 | + | + | ½ | 0 | ½ | 0 | ¼ | + |
| 75 | + | 18 | + | 10 | + | 10 | + | 4 | + | 2 | 0 | + | 1 | 0 | 1 | 0 | ½ | 0 |
| 80 | + | 21 | + | 15 | + | 15 | + | 6 | + | 3 | 0 | 0 | 1½ | 0 | 1½ | 0 | ¾ | 0 |
| 85 | + | 24 | + | 20 | + | 20 | 0 | 8 | 0 | 4 | 0 | 0 | 2 | 0 | 2 | 0 | 1 | 0 |
| 90 | + | 27 | + | 25 | + | | | 10 | 0 | 5 | 0 | 0 | 2½ | 0 | 2½ | 0 | 1¼ | 0 |
| 95 | + | 30 | + | | | | | 12 | 0 | 6 | 0 | 0 | 3 | 0 | 3 | 0 | 1¾ | 0 |
| 100 | + | 33 | + | | | | | 14 | 0 | 8 | 0 | 0 | 3½ | 0 | 3½ | 0 | 2 | 0 |
| 105 | 0 | 36 | 0 | | | | | | | 10 | 0 | 0 | | | | | | |
| 110 | + | 39 | + | | | | | | | 12 | 0 | 0 | | | | | | |
| 115 | 0 | 42 | 0 | | | | | | | 14 | 0 | 0 | | | | | | |
| 120 | 0 | 45 | + | | | | | | | 16 | 0 | 0 | | | | | | |
| | | 48 | 0 | | | | | | | | | | | | | | | |

+ indicates that bud transmitted disease.
0 indicates that bud did not transmit disease.

ease. Likewise, buds exposed at 42° C. for 36- and 42-minute intervals failed to transmit little peach, while those exposed for 39- and 45-minute intervals transmitted the disease. On the whole, the data recorded in table 4 indicate that little-peach virus is somewhat less resistant to heat than yellows virus. Tests with the 2 viruses at 48° C. were carried out at the same time and in exactly the same manner. Table 2 shows that yellows buds exposed for 8-, 10-, and 12-minute intervals all transmitted disease, and table 4 that little-peach buds exposed for the same periods of time all failed to transmit disease. Treatments for ½- and 1-minute intervals at 52° C. and 54° C., respectively, failed to cure yellows buds, while ½-minute treatments at these temperatures were sufficient to cure little-peach buds. At 56° C.,

TABLE 5.—*Thermal inactivation points of rosette and red-suture viruses in bud sticks*

| Rosette | | | | | | | Red suture | |
|---|---|---|---|---|---|---|---|---|
| Temperature 40° C. | | Temperature 50° C. | | | | | Temperature 50° C. | |
| Time in minutes | Effect | Time in minutes | Effect | Time in minutes | Effect | Effect | Time in minutes | Effect |
| 50 | + | 1 | + | 2 | + | + | 1 | + |
| 55 | + | 2 | + | 4 | + | + | 2 | + |
| 60 | + | 3 | + | 6 | + | + | 3 | 0 |
| 65 | + | 4 | + | 8 | + | 0 | 4 | 0 |
| 70 | + | 5 | + | 10 | 0 | 0 | 5 | 0 |
| 75 | + | 6 | + | 12 | 0 | 0 | 6 | 0 |
| 80 | + | 8 | 0 | 14 | 0 | 0 | 8 | 0 |
| 90 | + | 10 | 0 | 16 | 0 | 0 | 10 | 0 |
| 100 | + | 12 | 0 | 18 | 0 | 0 | 12 | 0 |
| 110 | + | 14 | 0 | 20 | 0 | 0 | 14 | 0 |
| 120 | + | 16 | 0 | | | | 16 | 0 |

+ indicates that bud transmitted disease.
0 indicates that bud did not transmit disease.

on the other hand, yellows virus was destroyed by a ¼-minute exposure, while little-peach virus was not.

The results obtained in an experiment with red-suture virus are recorded in table 5. Buds exposed for 3 minutes or longer at 50° C. were cured. Those exposed for 1- and 2-minute intervals were not cured. Results from 4 experiments with bud sticks affected by rosette are also shown in table 5. These experiments were carried out in exactly the same manner as were those reported in tables 2 and 4. Rosette virus was not destroyed in buds exposed at 40° C. for as long as 2 hours. At 50° C. it was destroyed by an 8-minute treatment but not by a 6-minute treatment in 2 experiments, and by a

10-minute treatment but not by an 8-minute treatment in another experiment. The data indicate that rosette virus is more resistant to heat treatment than are the viruses of the other 3 diseases. This conclusion was confirmed by several hot-room experiments, one of which will be described.

On February 6, 1934, 6 healthy young trees about 3 feet high were inoculated with rosette virus by inserting a bud in the stem of each tree at a point about 15 inches above the soil level. At the same time, 6 other trees were similarly inoculated, but with yellows buds. Six additional trees were left uninoculated to serve as controls. One week after the inoculations had been made, 3 trees in each set were transferred to the hot room where they were incubated for 2 weeks. At the end of this period the trees were returned to the greenhouse from which they had been taken. When observations were made, 100 days after treatment, 2 of the 3 treated rosette trees showed symptoms of rosette, while the other tree appeared healthy. All of the 3 trees inoculated with rosette and not treated showed marked symptoms of disease. The 3 that were inoculated with yellows and then treated appeared healthy, while the 3 inoculated with yellows and not treated were badly diseased. All of the noninoculated controls were healthy. No changes appeared in the trees of this experiment up to the time it was ended, a year later. Since all of the yellows trees and only 1 of the rosette trees were cured by the treatment, the experiment brings evidence that rosette virus is more resistant to heat than yellows virus.

It was never possible to cure trees of rosette after they had become diseased throughout. Such trees are very susceptible to injury by heat, and, even when exposed at the low temperature of the hot room, they died during or shortly after treatment. Rosette trees were readily cured when treated within from 1 to 3 weeks after infection.

## DISCUSSION

Yellows, rosette, little peach, and red suture are the only well-recognized virus diseases of plants that have been cured by heat treatments, but two other plant diseases suspected of belonging in the virus group have been so cured. When sugar-cane cuttings affected by the sereh disease of Java are immersed in water held at about 52° C. for 30 minutes to 1 hour, they are usually cured (6). The chlorotic-streak disease (12) affecting sugar cane in Hawaii, in Queensland (2), and in Java where it is known as the "fourth disease" has also been cured by a similar treatment at 52° C. for 20 minutes (13). So far as the writer is aware, neither of these diseases has been transmitted experimentally, and neither is definitely known to be caused by a virus. Wilbrink (15) concluded that sereh is probably due to an organism, since mosaic and similar diseases stand higher temperatures. Sugar-cane mosaic, which is transmitted by both mechanical and insect inoculations and

is definitely classified as a virus disease, has not been cured by heat treatments (4). Johnson (8) was unable to cure potato tubers of mosaic disease by heat. Blodgett (3) reports that heat treatments will not cure them of either mosaic or leaf roll, and Atanasoff (1) that it is evidently impossible to destroy the virus of stipple streak by heat treatments that do not injure tubers. Reddick and Stewart (14) and Fajardo (5) found that, when bean seeds affected by bean mosaic survived heat treatments, the virus also survived.

Finding that the 4 peach virus diseases under consideration yield to heat treatments, whereas several other virus diseases do not, suggests the possibility of a relationship between these diseases. Evidence indicating that yellows and little peach are closely related, but that rosette is distinct from both, has already been published (11). The fact that rosette virus is more resistant to heat treatments than virus of either little peach or yellows is in agreement with this evidence.

It is remarkable that yellows can be cured by a temperature below 34.5° C., and it would be interesting to know the minimum inactivation temperature. However, as temperature is lowered, the exposure period necessary for cure is greatly lengthened. This leads to practical difficulties that have not yet been overcome. Diseased trees held in the hot room for more than about a month become somewhat etiolated because of insufficient light. Such trees frequently die after they are returned to the greenhouse. Buds from sticks incubated in a water bath for long periods of time are apparently weakened, for many of them die after transplantation. On account of these difficulties, the minimum temperature of inactivation of yellows virus was not determined.

It has been suggested that viruses may be similar to the theoretical bearers of hereditary traits, which are known as genes. The experiments recorded here bring no evidence against this view, but they do show that the peach viruses that are inactivated by temperatures that cause no injury to peach tissues are less heat-stable than the genes of the peach, for the latter are apparently unaffected by exposures that readily inactivate virus.

A temperature of 35° C. (95° F.) is not unusual for the eastern United States during summer months, but such a temperature does not prevail over long periods of time except in the Southern States. This fact may afford a possible explanation for the southern limit of distribution of yellows in the Eastern States. It has long been known that at low elevations this limit lies only a short distance south of Washington, D. C., but extends far south of this latitude at high elevations. It thus follows an isotherm that passes across the eastern portion of southern Maryland and extends far to the south as it approaches the mountainous regions of Virginia and West Virginia, where summer temperatures are comparatively low. It is believed that the

temperatures that prevail south of this isotherm may be high enough and prolonged enough to inactivate yellows virus. Yellows occurs in the mountains of North Carolina and Georgia, but has never been reported from the lowlands of either State. Affected trees have doubtless been shipped into these regions.

Some of the reported experiments show the difficulty encountered in destroying yellows virus in the roots of potted trees. It was found to be much easier to cure tops than roots. This is presumed to have been due to cooling of roots by evaporation of water from the moist soil in which they were imbedded, rather than to any difference in resistance of virus in roots and in tops. The distribution of yellows virus in treated trees having healthy tops and diseased roots is strikingly like that of phony disease of peach. According to Hutchins (7), the phony virus is confined to roots. Heat-treatment experiments with yellows suggest that phony virus is absent from tops because of the high summer temperatures to which aboveground portions of orchard trees are exposed in Georgia where phony disease was studied.

No effort has yet been made to determine whether or not heat treatments will be useful in the control of virus diseases of peach. Orchard trees invaded by yellows virus frequently show no symptoms of disease until the end of the second season following infection. The danger of spreading yellows through the use of contaminated propagating material by nurseries has long been recognized. Valuable peach varieties developed in the eastern United States have not been introduced into California and other western states because of this danger. The evidence presented indicates that treated bud sticks could be used to introduce such varieties without risk of spreading the diseases under consideration.

Heat treatments for the cure of aerial portions of orchard trees affected by yellows, little peach, and red suture may prove practicable. If a diseased tree were covered by a canvas tent such as is used in fumigating, the air surrounding it might be raised, by means of an appropriate heating device, to a temperature high enough to cure the exposed top. The health of the tree might be sufficiently improved by destruction of virus in its top to justify the expense of treatment, especially if a 10- or 15-minute treatment proved effective. Such a method of applying heat would not destroy virus in roots, and it is not known whether a healthy top on a diseased root system would yield normal fruit. Moreover, the virus in the roots would spread into the top after a year or two and the treatment would then have to be repeated. It is not believed that such a method of treatment would prove successful for the cure of rosette, because of resistance of rosette virus to heat inactivation and the susceptibility of rosette trees to heat injury.

SUMMARY

Peach yellows was cured by incubating potted trees in a hot room held at a temperature that varied from 34.4° C. to 36.3° C. Cured trees assumed a normal habit of growth and produced healthy-appearing stems and leaves. The period of treatment necessary to effect a cure was longer for large than for small trees. Yellows virus was destroyed more quickly in small stems than in large ones and more quickly in tops than in roots that were imbedded in moist soil. Intermittent treatments did not prove effective. Dormant trees were cured by immersing them for about 10 minutes in a tank of water held at 50° C. The trees were not seriously injured by these treatments.

Yellows virus in buds was inactivated by immersing bud sticks in water held at a number of different temperatures. At 34.35° C. it was inactivated in from 4 to 5 days, at 38° C. in 11 hours, at 42° C. in 40 minutes, at 46° C. in 15 minutes, at 48° C. in 14 minutes, at 50° C. in from 3 to 4 minutes, at 54° C. in about 1 minute and 30 seconds, and at 56° C. in 15 seconds. The bud tissues were able to endure much longer treatments than were necessary to destroy virus.

Little peach, red suture, and rosette also were cured by exposure to heat. Little peach and red suture yielded to treatments that proved effective for the cure of yellows, but rosette was somewhat more refractory than the other three diseases.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH, PRINCETON, NEW JERSEY

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# SCLEROTIAL FORMATION IN RHIZOCTONIA SOLANI AS AFFECTED BY NUTRITIONAL AND OTHER FACTORS[1, 2]

W. B. ALLINGTON

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*Rhizoctonia solani* Kühn (*Corticium vagum* Burt) commonly overwinters in the sclerotial stage on potato tubers. Its attack on the young growing shoots and the cankering of the older stems causes more damage than do the sclerotia on the tubers. Nevertheless, rhizoctonia-infected tubers are decidely inferior to noninfected ones either for seed or for table purposes.

Variations in the number and size of sclerotia on potatoes have been observed commonly. Since there is nothing in the literature that adequately explains these variations, the present work was undertaken in an effort to determine the factors involved in the formation of sclerotia.

Matsumoto (6) noted that *Rhizoctonia solani* formed more sclerotia with maltose than with cellulose and also more with casein than with potassium nitrate. He concluded, therefore, that the carbon and nitrogen sources were influential. He also observed that the hydrogen-ion concentration greatly affected sclerotial development in *R. solani.* He concluded that the effect was due mainly to the differences in availability of the nutrients at the different hydrogen-ion concentrations. Monteith and Dahl (7) report that the type and color of sclerotia fluctuate with different degrees of acidity.

Raeder, Hungerford, and Chapman (8) believe that the moisture content and character of the soil influence materially the number and size of sclerotia. Hurst (4) recommends digging potatoes as early as possible because he believes that low temprature and moisture stimulate the development of sclerotia on the tubers.

Conditions causing sclerotia to form in other fungi may be similar to those affecting their development in *Rhizoctonia solani.* Ezekiel and coworkers (2) report more sclerotia with *Phymatotrichum omnivorum* (Shear) Duggar under conditions best suited for rapid and heavy vegetative growth.

It is improbable that staling processes lead to sclerotial development. Neither *Phymatotrichum omnivorum* (5) nor *Rhizoctonia solani* (1) pro-

[1] The results presented in this paper formed part of a thesis submitted by the author to the Graduate Faculty of the University of Nebraska in partial fulfillment of the requirements for the degree of Master of Arts. June 1935.

[2] The writer wishes to acknowledge his indebtedness to Dr. R. W. Goss for advice and criticism during the course of the investigation and in the preparation of the manuscript.

duce a staling effect in the medium, yet both organisms produce sclerotia abundantly.

Experimental evidence in support of these viewpoints is rather meager. The best explanations probably are those involving nutritive relationships.

## EXPERIMENTAL METHODS AND MATERIALS

The culture of *Rhizoctonia solani* used in these studies was a subculture of a single hyphal-tip isolate obtained from a germinating sclerotium removed from a potato grown in western Nebraska. The type of inoculum used in the culture was agar disks of a uniform size removed from the margin of a giant colony.

The media used were potato-dextrose agar and the following modification of Czapek's medium: magnesium sulphate 1.0 gm., potassium chloride 1.0 gm., potassium phosphate (di-hydrogen-) 2.26 gm., glucose 30.0 gm., urea 1.41 gm., agar 15.0 gm., water 1000.0 gm. The H-ion concentration, except when otherwise indicated, was adjusted to pH 6.2 after sterilization. Although filter-paper cones and sand with nutrient solutions were tried, the agar culture medium in Petri dishes proved most satisfactory and was used in all the experiments. Except where otherwise indicated, all cultures were incubated at 23° to 25° C.

## THE EFFECT OF THE POTATO TUBER ON SCLEROTIAL FORMATION

Small moist chambers, approximately 8.5 cm. in diameter and 4 cm. in height, and containing 75 cc. of 1.5 per cent water-agar, were used for the cultures. Just before the agar solidified, small potatoes were sterilized in alcohol and one was placed into each culture dish near one side. The potatoes were large enough to allow about half their surfaces to be exposed above the agar. After the agar solidified, it was inoculated with a disk from the margin of the potato-dextrose-agar giant culture on the side opposite the potato. The check consisted of 10 moist chambers, which were treated similarly, without the addition of the potatoes.

The organism grew equally in all the cultures until the hyphae were within 5 to 10 mm. of the potato. At this time a temporary inhibitory effect by the potato was noted. The mycelium surrounded the potato without coming closer than 5 mm. to it before the opposite side of the dish had been reached, then the hyphae advanced to the surface of the potato and in two days sclerotia formed on it or near by on the agar surface. In the check cultures a few sclerotia appeared on and near the point of inoculation only.

The experiment was performed again with like results. Each time 10 cultures with each treatment were employed. When the synthetic agar medium was used as the inoculum carrier, the results were similar except in

the checks. Here, the sclerotia did not form quite so close to the point of inoculation as when potato-dextrose agar was used and they were fewer and were deeply submerged in the agar.

A similar set of cultures was grown using both potato-dextrose and synthetic agar for inoculation disks but the tubers were removed from the cultures before contact with the fungus. Although this experiment was not repeated, the results were very consistent in the 10 cultures employed.

The sclerotia invariably appeared near the source of inoculum in all the cultures. When potato-dextrose agar was used for the inoculum carrier, they appeared closer to the inoculation disk than when synthetic agar was used.

From these results it is obvious that the potato tuber influences the formation of sclerotia. Although these experiments show an inhibitive effect by the potato upon the fungus, it is improbable that this or any toxic effect from the potato stimulates sclerotial development. If some inhibitory agent were present that would cause sclerotia to form, they would have formed also near the point of inoculation as in the checks. An inhibitory substance did not remain in the agar after the removal of the potato, as shown by the unaffected progress of growth through this area.

The medium used in the cultures consisted of distilled water and agar only, leaving two isolated regions where added nutrients were available, namely, the inoculation disk and the potato. Since these regions were the seat of sclerotial formation, it appears that nutrition was a factor concerned with sclerotial development. It was interesting to note that the hyphae had to come in contact with the tuber if the latter was to affect sclerotial formation. It is possible, therefore, that the removal of food substances from the potato facilitates sclerotial development.

In a series of greenhouse experiments involving temperature studies, sclerotia were never observed to form upon buried foreign objects, such as smooth or frosted glass or porous clay surfaces. Many dormant potatoes were heavily covered with sclerotia while buried in the same soil. It appears, then, that the potato does more than merely furnish a surface where sclerotia may form.

## THE EFFECT OF CARBON AND NITROGEN UPON THE FORMATION OF SCLEROTIA

### Source of Carbon

The effect of different kinds of carbon sources was studied by means of Petri-dish cultures on agar. The carbon compounds substituted for glucose in the synthetic medium were sucrose, potato starch, glycerol, and lactic acid. Equivalent amounts of carbon to that in the original synthetic medium were used. Two different nitrogen sources were employed, *i.e.*, for inorganic,

ammonium nitrate; for organic, urea. The nitrogen compounds were used in concentrations equivalent to the nitrogen in the synthetic medium.

The experiment was repeated 3 times and with like results. The differences in growth rate and sclerotial formation are shown in table 1. The

TABLE 1.—*Relative growth of mycelium and formation of sclerotia by* Rhizotonia solani *with different sources of carbon and nitrogen*

| Compounds furnishing carbon[a] | Ammonia nitrate | | Urea | |
|---|---|---|---|---|
| | Diameter of colonies after 3 days | Sclerotial formation after 10 days | Diameter of colonies after 3 days | Sclerotial formation after 10 days |
| | *mm.* | | *mm.* | |
| Glucose | 58 | Abundant[b] | 23 | Medium |
| Sucrose | 55 | Abundant | 40 | Medium |
| Potato starch | 60 | Abundant | 30 | Medium |
| Glycerol | 50 | Medium | 25 | Slight |
| Lactic acid | 50 | None | 15 | None |

[a] The amount of carbon in each of the compounds was equivalent to the amount in the original synthetic medium, p. 832.

[b] The word "abundant" refers to both size and number of sclerotia.

results shown in this table were taken from a representative set of cultures in a series of replications. The data show that the organism can use glucose, sucrose, and potato starch very readily for sclerotial formation. Glycerol and lactic acid are definitely less favorable. A marked difference is shown between the organic and inorganic nitrogen. Growth and the formation of sclerotia were much greater with ammonium nitrate. Sclerotia appeared earliest in those cultures that ultimately had the most sclerotia.

*Amount of Carbon.*—The effect of different amounts of carbon was studied with glucose as the carbon source. It was found in preliminary work that the transfer of mycelium to a starvation medium resulted in a very poor initiation of growth. Therefore, a different procedure was adopted in the preparation of the cultures. The various media, which were dilutions of the original synthetic medium with the same per cent of agar present, were pipetted into Petri dishes in equal amounts. After solidification, round disks 40 mm. in diameter were aseptically removed from the center of each dish. The resulting vacant areas were subsequently filled with a small amount of the synthetic medium and then inoculated. Any differences resulting from unequal growth initiation were greatly reduced before the hyphae reached the test medium.

The experiment was repeated, involving many cultures that gave consistent results. Table 2 shows the average growth and sclerotial formation of one series of cultures. Diffusion of the carbohydrate from the central

TABLE 2.—*Relative total growth and sclerotia formation by* Rhizoctonia solani *with decreasing amounts of carbohydrate, nitrogen, and total nutrients*

| Concentration of variant | | Average diameter of colonies after 3 days | Sclerotial development after 10 days |
|---|---|---|---|
| | *Per cent* | *mm.* | |
| Glucose | 3.00 | 66 | Very slight |
| " | 1.50 | 67 | Slight |
| " | 0.75 | 65 | Medium |
| " | 0.35 | 69 | Medium |
| " | 0.20 | 69 | Abundant[a] |
| " | 0.00 | 70 | Abundant |
| Urea | 0.140 | 75 | Slight |
| " | 0.070 | 68 | Slight |
| " | 0.030 | 69 | Slight |
| " | 0.015 | 66 | Very slight |
| " | 0.007 | 66 | Very slight |
| " | 0.000 | 62 | None |
| Total Nutrients | 100.0 | 73 | Very slight |
| " " | 50.0 | 74 | Medium |
| " " | 25.0 | 75 | Slight |
| " " | 12.0 | 69 | None |
| " " | 6.0 | 70 | None |
| " " | 0.0 | 65 | None |

[a] The word "abundant" refers to both size and number of sclerotia.

portion of the culture undoubtedly took place. Nevertheless, the effect of smaller amounts of carbon was clearly demonstrated. There were no significant differences in the growth response of the mycelium within these small culture dishes with their varying amounts of carbohydrate, but the number of sclerotia greatly increased when the carbohydrate was diminished. The cultures having the most sclerotia also were those in which they formed first.

The sclerotia never appeared in these cultures until the entire volume of agar in the dish had been covered with fungous hyphae. In an attempt to eliminate this condition, large moist chambers 17 cm. in diameter were later used for culture dishes. Even in these giant culture dishes, regardless of the nutrient conditions, sclerotia never appeared before all the agar was covered.

Interesting results were obtained when varying amounts of glucose were added to the medium in the large moist chambers. A portion of the basic synthetic medium was substituted in the center of the moist chambers containing the test media, the same as with the above mentioned Petri-dish cultures. When the hyphae reached the periphery of the moist chambers,

sclerotia soon appeared. The position of these sclerotia was particularly interesting. In all the cultures containing glucose with a concentration of 0.35 per cent or more, the sclerotia formed upon the edge of the moist chamber or very close to it. In the cultures containing 0.20 per cent, and in those with no glucose, the sclerotia formed in a ring $\frac{3}{4}$ and $\frac{1}{2}$ the way out, respectively, from the center of the dish.

Carbohydrate concentration is thereby shown to affect sclerotial formation when the nitrogen and other nutrients are constant. There is yet no means of differentiating between the effect of total concentration of carbohydrate and the relationship between carbohydrate and nitrogen. Subsequent experiments deal in part with this phase of the problem.

### Source of Nitrogen

The following experiment was conducted to determine any further relationships between the source of nitrogen and the development of sclerotia.

The basic synthetic medium was used with the various nitrogen compounds substituted for urea. The concentration of the nitrogen compounds was determined by using amounts of nitrogen equivalent to that in the basic synthetic medium. These nitrogenous compounds were: urea, asparagine, calcium nitrate, ammonium nitrate, and sodium nitrate. The test medium was pipetted in equal amounts into Petri dishes. All inoculations were made directly upon this medium at the center of each dish.

Variation in growth rate was insignificant with the different sources of nitrogen tested. On the other hand, sclerotial formation was greatly influenced by the kind of nitrogen (Fig. 1, A). There was variation not only in numbers but in size and character of the individual scelrotia. The cultures having the most sclerotia also were the first to have them appear.

The use of urea resulted in the greatest number of sclerotia; but they were very small, and the majority were submerged in the agar. Close examination of the cultures (Fig. 1, A) disclosed innumerable minute sclerotia in the dish containing urea. That containing asparagine showed several large black sclerotia, accompanied by a great many smaller ones. Many of the smallest were submerged and were similar to those in the dish containing urea. The sclerotia in the dish containing calcium nitrate were all upon the surface of the agar, but the majority were exceedingly small. They tended to coalesce, forming large irregular black blotches upon the agar surface. In the dish containing ammonium nitrate there were fewer sclerotia, most of them were superficial, of medium size, and typically round and hard. The sclerotia in the culture containing sodium nitrate were mostly small and comparatively few.

After sclerotial formation the medium was removed from the culture dishes, melted and the pH was determined. The results shown in table 3

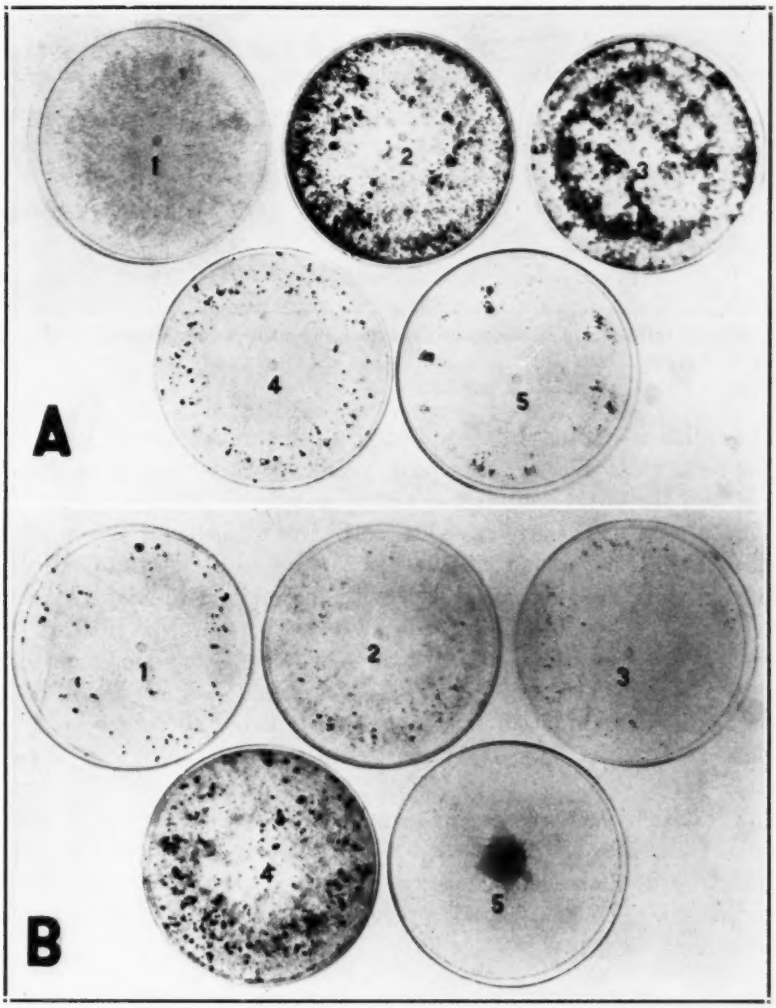


FIG. 1. Cultures of *Rhizoctonia solani*. A. Differences in sclerotial formation with various sources of nitrogen: 1, Urea; 2, asparagine; 3, calcium nitrate; 4, ammonium nitrate; 5, sodium nitrate. B. Differences with various H-ion concentrations. 1, pH 4.0; 2, pH 5.0; 3, pH 6.0; 4, pH 7.0; 5, pH 8.0.

clearly indicate that *Rhizoctonia solani* is able to change the pH of its substratum. The direction and extent of change is dependent upon the nitrogen source.

The results obtained by using different nitrogen sources probably cannot be explained by the effect of pH. Sodium nitrate and calcium nitrate cause the pH to rise to somewhat similar values, yet sclerotial formation

TABLE 3.—*Changes of H-ion concentration of the medium by* Rhizoctonia solani *with different sources of nitrogen*

| Nitrogen source[a] | Final pH[b] |
|---|---|
| | *pH* |
| Urea | 6.1 |
| Asparagine | 6.0 |
| Calcium nitrate | 6.8 |
| Ammonium nitrate | 4.2 |
| Sodium nitrate | 6.9 |

[a] The concentration of nitrogen used in all compounds was equivalent to that in the basic synthetic medium.

[b] The initial pH of all media was 6.2.

is not similar with the two compounds. Ammonium nitrate and sodium nitrate, both, yield sclerotia sparingly, yet the former leads to an extremely low pH and the latter to a higher pH.

*Concentration of Nitrogen.*—The following experiment shows the relationship between the amount of nitrogen present and sclerotial development.

The synthetic medium, combined with the method previously described of surrounding the full nutrient medium with the test media in Petri dishes, was used. The test media consisted of the synthetic medium with varying amounts of urea substituted as the single nitrogen source. Equal amounts of the media were pipetted into the culture dishes to insure comparable growing conditions. The experiment was repeated once with consistent results.

The results of the experiment are shown in table 4. Growth rate and sclerotial formation were decreased simultaneously when the nitrogen was decreased. When nitrogen was absent from the medium there was no sclerotial formation. The results are the reverse of those obtained with decreasing carbohydrates, as shown in table 2.

## THE EFFECT OF A DECREASE IN TOTAL NUTRIENTS UPON SCLEROTIAL FORMATION

A decrease in carbohydrate has been shown to stimulate sclerotial development, while a decrease in nitrogen inhibits sclerotial formation. It was then necessary to determine how the organism reacts when all the nutrients were decreased.

The usual type of Petri dish culture was used, *i.e.*, the test medium surrounded the usual basic synthetic medium. A large quantity of the basic synthetic medium was made and diluted in distilled water to give the following percentages of the original concentration: 100, 50, 25, 12, and 6 per cent. One series of plates contained no added nutrients in the test medium. Inocu-

lation was made as usual upon the medium in the center of the dish, and the mycelium grew out over the test medium. The experiment was repeated once, with 5 cultures for each test medium.

The results, which clearly indicate the effect of decreasing nutrients, are shown in table 2. In 10 days sclerotia had not appeared at concentrations below 25 per cent. Evidently, sclerotial formation is not a simple starvation phenomenon. Previous tests have given more sclerotia with low carbohydrate or high nitrogen. It will be observed in table 2 that sclerotial formation first increased with lower nutrients and then decreased sharply. The increase with the initial reduction in nutrients of 50 per cent was probably due to the effect of decreasing the carbohydrate. The latter decrease in sclerotial development was the effect of lower nitrogen.

### THE EFFECT ON SCLEROTIAL DEVELOPMENT OF ADDING NUTRIENTS TO STARVED CULTURES

After determining that sclerotial development was affected by nutritional factors, it was decided to add certain nutrients to starved cultures and note the reactions of the fungus.

A starvation medium consisting of 1.5 per cent agar in distilled water was used in large moist chambers, 5 cm. deep and 17 cm. in diameter. A small disk of the basic synthetic medium was placed in the center of each culture, in place of the starvation agar, and inoculated. Preliminary experiments with the same type of cultures showed that, by the use of a small disk of synthetic medium in the center, the organism would grow out over the starvation medium but that it never formed sclerotia. At the time of inoculation, the test substances were added near the periphery of the moist chamber. The following were placed in the culture dishes: sterile potatoes, aseptic potato disks, glucose in agar, basic synthetic agar, ammonium nitrate in agar, urea in agar, and a check composed of distilled water and agar only. When the nutrient compounds were added to an agar medium, the concentrations were the same as in the basic synthetic medium. The aseptic potato disks were taken from mature potatoes within one centimeter of the periderm. The agar and potato disks were 12 mm. wide and 3 mm. thick. The sclerotia were abundant around the potato disk, but occurred very sparsely around the disk containing urea. In the other cultures sclerotia formed on the potato and around the ammonium nitrate disk. A decided stimulation to mycelial growth was evident with all the nitrogen compounds.

The cause of sclerotial development is not simple starvation. Unless the fungus colony could grow over the entire agar surface before the food supply became sufficiently exhausted to stop growth, the sclerotia failed to appear. In this experiment the food supply was exhausted to that extent and the value of certain substances in the formation of sclerotia was demonstrated. The results show conclusively that high carbohydrate is of no

effect but that the addition of nitrogen has a decided stimulatory effect upon sclerotial development. The results conform very favorably to those of previous experiments, which showed that high nitrogen and low carbohydrate gave the most sclerotia.

### THE EFFECT OF THE H-ION CONCENTRATION OF THE MEDIUM ON SCLEROTIAL FORMATION

It has been shown that the H-ion concentration of a medium is sometimes changed during the growth of *Rhizoctonia solani* (3). The following experiment was conducted to determine the effect of different initial H-ion concentrations upon sclerotial development.

The synthetic medium was adjusted to the desired pH value by the addition of HCl or NaOH. The following pH values were employed: 4.0, 5.0, 6.0, 7.0, and 8.0. The medium was pipetted into Petri dishes and inoculated.

The results are shown in figure 1, B. *Rhizoctonia solani* was able to grow at every pH value tested, although the growth rate was extremely low at a pH 8.0. Maximum growth occurred at pH 7.0 and sclerotia were formed in abundance. Fewer sclerotia developed at 4.0, 5.0, and 6.0 and there was very little difference in these sets. At pH 8.0 the mycelium presented a peculiar appearance. The hyphae were densely matted together, but sclerotial hyphae were not observed.

Growth and sclerotial development of *Rhizoctonia solani* were favored by neutral or acid conditions. Very little growth was possible at pH values above 7.0.

### THE EFFECT OF TEMPERATURE ON SCLEROTIAL FORMATION

It had been suggested (4) that low temperature may cause *Rhizoctonia solani* to form sclerotia on the potato tuber in the fall. If low temperature affects sclerotial formation in the soil, it is probable that a similar reaction would occur in pure culture and the following experiments were outlined to determine these two points.

#### Effect in Pure Culture

Petri dishes containing potato-dextrose agar were inoculated and incubated at 25° C. for one day. At this time the colonies were approximately 20 mm. in diameter. Five cultures were then placed in incubators at each of the following temperatures: 5°, 10°, 15°, 20°, and 25° C. Growth measurements were taken daily and the time and character of sclerotial formation were carefully noted.

The rate of growth was considerably less at lower temperatures as reported by Monteith and Dahl (7). At 25° C., the highest temperature

to which the cultures were exposed, the growth was the greatest. At 5° the organism was unable to grow, but remained alive during the exposure.

Sclerotia appeared first in the cultures subjected to the higher temperatures, probably due to the fact, previously stated, that they do not form until the medium is covered with mycelium.

When cultures held at 5° C. for 10 days were transferred to a temperature of 25° C., normal growth was resumed and continued until the culture dish was covered, then sclerotia formed predominately at the center of the dish. This region was precisely the same as that covered by mycelium during exposure to low temperature. When cultures were held at 5° for 20 days, no sclerotia appeared until exposure to higher temperatures. Microscopic examination of the cultures at the time of transfer from 5° to 25° revealed no tendency toward sclerotial formation.

The peculiar clustering of sclerotia in the central region of each culture probably resulted from different metabolic activity of the fungus while at low temperature an activity that caused different nutritive conditions to prevail.

### Effect in Soil

Greenhouse soil was inoculated with a heavy suspension of *Rhizoctonia solani* mycelium and then mixed well before potting. Sclerotia-free potatoes were disinfected with alcohol and buried for 20 days in the soil which was kept at an optimum moisture with both high and low temperature. At the termination of the experiment the potatoes were dug, washed, and graded for the presence of sclerotia.

The data obtained were inconclusive and further repetition of the experiment is required. However, it was of interest to note that sclerotia formed under these conditions. In some cases very heavy infection was obtained. It is possible, therefore, that potato seed-pieces, which are healthy when planted, may have abundant sclerotia on them if removed from the soil 20 days later for examination.

## DISCUSSION

The results indicate that a number of factors influence sclerotial development in *Rhizoctonia solani*. These factors may act collectively or individually to enhance or to inhibit the effect of other factors.

Sclerotia did not form in Petri-dish cultures until the growth of the organism was physically limited. This phenomenon occurred regardless of whether dishes 10 cm. or 17 cm. in diameter were used. Apparently, therefore, the age of the cultures was of little significance in sclerotial formation.

Sclerotial formation is affected greatly by differences in nitrogen source. Although the extent and direction of the change of H-ion concentration in

the medium is influenced by the nitrogen source, the variations of sclerotial development cannot be explained on this basis. It is improbable that the changes in H-ion concentration in the medium affect the solubilities of the nitrogenous compounds sufficiently to account for the differences in sclerotial development.

*Rhizoctonia solani* can readily utilize glucose, sucrose, and potato starch. These substances sustained good growth and sclerotial development with no detectable differences. Matsumoto (6) has found the organism capable of digesting cellulose and other complex carbohydrates.

Sclerotial formation decreased when less than 50 per cent of the original amount of nutrients were added to the culture medium. When slight reductions were made, however, sclerotial development increased. After the agar is completely covered mycelial growth is slowed up considerably. At this time the assimilation of nitrogen is at a minimum, while the assimilation of carbohydrate is still taking place at a relatively high rate. The demand for carbohydrate is greater than for nitrogen, hence the decrease in carbohydrate is the first to have an effect. The increase in sclerotial development due to high nitrogen can be explained on the same basis. Cultures with high nitrogen invariably produce a heavy mycelial growth and demand more carbohydrates for maintenance.

It has been found that greater sclerotial infection occurs on potatoes in western Nebraska that were planted early and matured early during periods of high temperatures.[3] This, along with sclerotial development upon mature tubers late in the fall, indicates a correlation between sclerotial formation and maturation of the potato or with higher temperature conditions. Werner (9) has analyzed potato tubers at different stages of maturity and reports an increase in the percentage of total nitrogen with maturation. This increase in nitrogen might cause an increase in sclerotial development just as it did in pure culture. Since pure-culture studies have shown more sclerotia at higher temperatures, the above mentioned results might be explained on that basis. The appearance of heavy infections in the fall, however, tends to indicate that maturity has more influence.

Since low temperature retards sclerotial formation in pure culture, it is doubtful if it hastens the process in nature. The conclusions of many who have observed the increased sclerotial infection upon potatoes when they are harvested late in the season, indicate that temperature is considered to be the causal factor. The host plant has never been considered in an explanation of this phenomenon. Since nutrition plays a part in sclerotial formation and the potato may be penetrated by the fungus, the host must be considered.

Sclerotia upon potatoes are more common near the stem end of the tubers. There are two possible explanations of this phenomenon. The

[3] Unpublished data by R. W. Goss, Nebraska Agr. Expt. Sta.

periderm of the potato near the stem end is the oldest and has been exposed the longest to possible infection. This part of the potato is more mature and differs chemically from the other parts. Therefore, if maturity affects sclerotial development, a predominance of sclerotia might be expected near the stem end.

Growth and sclerotial development have been shown to be favored by a pH near 7.0. The number and size of sclerotia varied greatly at different pH values. In many cases sclerotial variations in different localities may be due to the soil reaction and other conditions and not to different strains of the organism.

A careful investigation of the effect of staling upon sclerotial development has not been made. However it has been shown by Allen and Haenseler (1) that *Rhizoctonia solani* does not produce substances in the medium that are autotoxic. Since the medium employed in this investigation was very similar to that used by Allen and Haenseler, the author has disregarded the staling factor. The cotton root-rot fungus produces sclerotia, yet has no staling effect upon its medium (5). It is very improbable, therefore, that staling is a factor in causing sclerotial development.

The effect of moisture upon the formation of sclerotia has not been investigated. Field observations are somewhat contradictory. An investigation of this phase of the problem is desirable.

If we are to determine the actual cause of sclerotial development, we must conceive of an intricate set of conditions, which, collectively, have an influence in one direction. The complexity of this mechanism appears at present to be very great.

## SUMMARY

The effect of various nutritional factors, temperature, and H-ion concentration of the substratum upon sclerotial development of *Rhizoctonia solani* Kühn was studied in pure culture. Other factors, commonly attributed to be influential in sclerotial development, are discussed.

Sclerotia never formed before all the agar in the culture dish has been covered by the colony.

The potato tuber itself had an influence upon sclerotial formation. This influence may be enhanced by maturation due to the increased nitrogen content of the tuber at that time.

Sclerotia formed most readily with relatively low carbohydrate and high nitrogen.

Extreme variations in sclerotial development were evident with different nitrogen sources. The nitrogen compounds in the order of decreasing sclerotial formation were: calcium nitrate, asparagine, ammonium nitrate, urea, and sodium nitrate.

No differences in sclerotial development were observed with glucose, sucrose, or potato starch as the carbon source. Glycerol and lactic acid were not utilized readily by the fungus.

A small decrease in total nutrients caused increased sclerotial development; a further decrease resulted in decreasing sclerotia.

*R. solani* was able to change the H-ion concentration of its substratum, the direction and extent of change depending upon the nitrogen source. This does not account for the variations in sclerotial development with different nitrogen sources.

An H-ion concentration near 7.0 favored growth and sclerotial formation. The organism was quite tolerant to acid but not to alkaline conditions.

Temperature changes did not stimulate sclerotial development in pure culture.

In general, sclerotial development paralleled vegetative growth.

Sclerotia formed upon healthy seed potatoes buried in inoculated soil. It is possible, therefore, to have healthy seed-pieces become infected after planting.

UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

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# THE TOLERANCE OF ERYSIPHE POLYGONI AND CERTAIN OTHER POWDERY MILDEWS TO LOW HUMIDITY[1]

CECIL E. YARWOOD[2]

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## METHODS

Conidia for germination and inoculation tests were taken from mildewed greenhouse plants during the light portion of the day, unless otherwise mentioned. To insure a more uniform distribution of the conidia on the test substrata, the slides, Petri dishes, leaves, or plants were placed in the bottom of a large can and conidia were dusted on them from above. Unless otherwise mentioned the dry slides with conidia were placed in Petri-dish moist chambers in the diffuse light of the laboratory for germination tests. Germination percentages always are based on counts of 200 or more conidia, including shrivelled ones. Shrivelled conidia, evidenced by wrinkled walls, loss of turgor and vacuolation, and decrease in size and transparency, were commonly present in small numbers in any sample of conidia from field or greenhouse plants, and they did not germinate. When subjected to low humidity many normal conidia became shrivelled either without germination or during germination. Hence, counts of shrivelled conidia include those that were shrivelled before being placed to germinate, as well as those shrivelled during the germination interval. When conidia were placed at 100 per cent relative humidity, the number of conidia shrivelled during germination would be negligible, while, at low humidity, the number of such conidia might be considerable.

To produce different relative humidities, solutions were prepared with C. P. sulphuric acid and distilled water; and these solutions are assumed to produce the relative humidities tabulated by Stevens[3], except for the concentrated acid (S. G. 1.84), which was estimated from Stevens' figures to give a relative humidity of approximately 0. Dehydration of the air of the chambers and of the experimental leaves and spores weakened the sulphuric acid solutions. Consequently, the relative humidities did not remain at the theoretical values they had at the time the solutions were prepared. As the amount of acid was large in comparison with the amount

[1] Contribution from the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture; the Botany Department, Purdue University Agricultural Experiment Station; the Department of Plant Pathology, University of Wisconsin; and the Division of Plant Pathology, University of California.

[2] The writer acknowledges his indebtedness to Dr. M. W. Gardner for assistance during the course of the work and in the preparation of the manuscript.

[3] Stevens, N. E. A method for studying the humidity relations of fungi in culture. Phytopath. 6: 428–432. 1916.

of experimental material, however, this source of error is not considered significant in these studies. The stillness of the air in the experimental humidity chambers might be expected to allow considerable local variation in relative humidity within the chambers and a higher humidity near the leaf and fungus surfaces. But, since, in two germination tests in which rapid air circulation within the chamber was provided, the results were similar to those where no artificial circulation was provided, local variations in the relative humidity within the chambers are not considered to be important in the small chambers.

The laboratory environment used extensively in these tests has not been controlled, but has remained fairly constant. Cultures were protected from direct sunlight, but were maintained in strong diffuse light near an east window. The temperatures in the laboratory during these tests have been observed to vary between the limits of 19° and 24° C. and numerous measurements of relative humidity during the day and night indicate that this has remained between the limits of 25 and 55 per cent, with an average of about 44 per cent.

### THE EFFECT OF HUMIDITY ON THE GERMINATION OF CONIDIA

Results of 3 tests to measure the gross relation between humidity and the germination of conidia of clover, barley, oat, and sunflower mildews are given in table 1. Germination resulted when the conidia were on the surface of water, suspended in water, on dry glass at 100 per cent relative humidity, and on dry glass at about 0 relative humidity, and only with barley and sunflower mildew conidia at about 0 relative humidity was the amount of germination significantly lower than that under the best conditions provided in these limited tests. Relative humidities intermediate between 0 and 100 per cent have been extensively tested; but, as would be expected, it was difficult to measure differences in germination due to even large differences in relative humidity. As the objection has been raised that the circulation of air in the stationary Petri-dish germination chambers used in these tests might be inadequate to insure a thorough distribution of air at the relative humidity determined by the humidity-regulating solution, other tests were made in which the conidia were on the inner surface of Petri-dish lids, 15 cc. of humidity-regulating solution was placed in each dish, the dishes were sealed with rubber bands, and then placed on an inclined rotating table, so that the humidity-regulating solution was kept continuously agitated. Results in such chambers were similar to those in which no such precautions for humidity regulation were taken, and 47 and 87 per cent, respectively, germination of clover mildew conidia resulted over C. P. sulphuric acid in 2 successive tests in such chambers.

TABLE 1.—*Percentage germination of conidia resulting under different conditions of humidity for 6 hours at about 22° C.*

| Location and organism | Conidia on the surface of water | Conidia at 100 per cent relative humidity on dry glass | Conidia suspended in water | Conidia at 0 relative humidity on dry glass |
|---|---|---|---|---|
| | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| Lafayette, Indiana, 1930 | | | | |
| *Erysiphe polygoni* from red clover | | 56 | | 26 |
| Madison, Wisconsin, 1933 | | | | |
| *E. polygoni* from red clover | | 76 | | 47 |
| *E. graminis* from barley | | 24 | | 2 |
| Berkeley, California, 1935 | | | | |
| *E. polygoni* from red clover | 65 | 86 | 57 | 63 |
| *E. graminis* from oats | 59 | 39 | 41 | 25 |
| *E. cichoracearum* from sunflower | 24 | 9 | 13 | 1.5 |

While conidia of clover mildew will germinate at low relative humidities, they may be killed by only a short period of drying under certain conditions. Conidia attached to conidiophores on mildewed, but otherwise normal, leaves retain their germinability for several days under field conditions; but, if the mildewed leaves are detached from the plant and allowed to dry out, the conidia soon are killed. A number of mildewed leaves were cut from field plants at Madison, Wisconsin, at 7 p. m., July 9, and hung in the field to dry. Spore mounts were made from these leaves at intervals thereafter, and the germinability of the conidia and the number of shrivelled conidia were determined. The results, given in table 2, indicate that most of the conidia were dead within 36 hours.

In certain cases the germinability of conidia may be favored by drying. Several tests with clover mildew have given unsatisfactory results, but the results of 3 tests with bean mildew appear conclusive and are given in table 3. Conidia for these tests were produced in the laboratory. For the flask cultures, greenhouse plants with 2 true leaves were excised above the soil level and the stems placed in flasks of tap water and the leaves held in the laboratory environment. Such excised plants formed roots in the water, regenerated new leaves and supported a luxuriant growth of mildew which showed a diurnal cycle[4] characteristic of *Erysiphe polygoni*. The

[4] Yarwood, C. E. The diurnal cycle of the powdery mildew *Erysiphe polygoni*. Jour. Agr. Res. **52**: 645–657. 1936.

TABLE 2.—*Germination of conidia from mildewed clover leaves cut from field plants and dried in the field. 1933, Madison, Wisconsin*

| Time when leaves were cut from plants | Time of preparing spore mount | Period during which excised leaves were exposed to field conditions | Germination interval in moist chambers at 25° C. in dark | Spores germinated | Spores shrivelled |
|---|---|---|---|---|---|
| | | *Hours* | *Hours* | *Per cent* | *Per cent* |
| 7 p. m. July 9 | 7 p. m. July 9 | 0 | 12 | 56 | 5 |
| " | 7 a. m. " 10 | 12 | 14 | 68 | 3 |
| " | 10 " " " " | 15 | 12 | 50 | 4 |
| " | 1 p. m. " " | 18 | 10 | 22 | 8 |
| " | 7 " " " " | 24 | 12 | 1.8 | 87 |
| " | 7 a. m. " 11 | 36 | 11 | 0.3 | 89 |

dish cultures referred to in table 3 were mildew-infected excised leaves on 5 per cent sucrose solution in Petri dishes. Hence, the conidia from the flask cultures were produced at about 40 per cent relative humidity, while those from the dish cultures were produced in a saturated atmosphere. Yet, the general results with conidia from these two sources were similar. In the manipulation of each of these tests, 4 slides were dusted at about noon with conidia from each spore source. One slide was placed immedi-

TABLE 3.—*The effect of relative humidity and drying on the germination of conidia of E. polygoni from bean leaves at about 22° C., Berkeley, California, 1935*

| Treatment given the conidia | January 3 | | January 5 | | January 7 | |
|---|---|---|---|---|---|---|
| | Dish culture germination | Flask culture germination | Dish culture germination | Flask culture germination | Dish culture germination | Flask culture germination |
| | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| 100 per cent R. H. | 9 | 0 | 13 | 8 | 21 | 14 |
| 0 R. H.[a] | 7 | 27 | 30 | 17 | 30 | 11 |
| Dried 2 hours, then at 100 per cent R. H. | 42 | 13 | 48 | 15 | 83 | 50 |
| Dried 8 hours, then at 100 per cent R. H. | 83 | 20 | 63 | 17 | 41 | 23 |

[a] Most germinated and nongerminated conidia had shrivelled after 24 hours under these conditions.

ately in a Petri-dish moist chamber at 100 per cent relative humidity; one was placed at 0 relative humidity (in a Petri dish with C. P. sulphuric acid); one was allowed to dry for 2 hours on the laboratory table and then transferred to 100 per cent relative humidity; and another was allowed to dry for 8 hours on a laboratory table and transferred to 100 per cent relative humidity. Germination was counted on all slides at 24 hours after preparing the mounts. The results show that drying stimulated germination in most cases and that germination over sulphuric acid was greater than that over water, though most of the conidia over sulphuric acid for 24 hours were severely shrivelled. Conidia dried for 2 or 8 hours showed higher germination in most cases than those at 0 relative humidity continuously. Conidia produced in dish cultures at approximately 100 per cent relative humidity showed higher germination in these tests than did those produced in flask cultures at about 40 per cent relative humidity.

### THE EFFECT OF TEMPERATURE ON THE TOLERANCE OF CONIDIA TO LOW HUMIDITY

Although low relative humidity does not always reduce the amount of germination, it always causes considerable shrivelling of the germinated and nongerminated conidia, and this desiccation action is more pronounced at high temperatures. The results of a test of the relation of temperature to desiccation injury of clover mildew and mustard mildew is given in table 4. In this test the percentage of shrivelled conidia in the mounts over sulphuric acid was progressively greater as the temperature was increased, until at 22° C. all the conidia were shrivelled in the 30-hr.

TABLE 4.—*The effect of temperature on the tolerance to low humidity of the conidia of E. polygoni from red clover and from mustard. March 1935, Berkeley, California*[a]

| Temperature of germination | Relative humidity in germination chamber | Clover mildew | | Mustard mildew | |
|---|---|---|---|---|---|
| | | Germination | Shrivelled conidia | Germination | Shrivelled conidia |
| °C. | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| 13 | 100 | 19 | 0 | 40 | 14 |
| | 0 | 37 | 20 | 0 | 48 |
| 16 | 100 | 37 | 0 | 45 | 9 |
| | 0 | 40 | 96 | 4 | 72 |
| 19 | 100 | 41 | 0 | 46 | 10 |
| | 0 | 47 | 95 | 3 | 95 |
| 22 | 100 | 63 | 0 | 41 | 13 |
| | 0 | 65 | 100 | 0.5 | 100 |

[a] Conidia of clover mildew from greenhouse potted plants. Conidia of mustard mildew produced on excised leaves. Conidia placed in darkness and germination counted after 30 hours.

period of the test. The slightly greater germination of clover mildew conidia at the lower humidity at each temperature is of interest. The test also shows that conidia of clover mildew are more tolerant to low humidity than are the conidia of mustard mildew (also *Erysiphe polygoni*). A deduction from this and other tests not shown in the table is that conidia are more sensitive to the desiccative action of sulphuric acid after germination than before. For instance, in one test at 0 relative humidity for 24 hours, 80 per cent of the total conidia had shrivelled, 98 per cent of the germinated conidia had shrivelled, while 67 per cent of the nongerminated conidia had shrivelled.

### THE ACTION OF HOST LEAVES IN PROTECTING CONIDIA FROM THE INJURY CAUSED BY DESICCATION AND HIGH TEMPERATURE

As already indicated, while conidia may germinate on glass slides at the lowest relative humidities tested, they are soon shrivelled and killed under these conditions, especially at high temperatures. With conidia on host leaves this injurious action of low humidity is much less apparent, and continued luxuriant development of clover mildew from inoculation to sporulation has occurred at the low relative humidity produced in closed chambers by C. P. sulphuric acid. The protective action of the turgid leaf in reducing the injurious action of low humidity on the fungus was apparent also when the mildew colonies on leaves were subjected to drying at high temperatures in different ways. The results of one test given in table 5, show that when conidia are exposed on dry slides or on detached

TABLE 5.—*The effect of exposure to 35° C. and 50 per cent relative humidity on the subsequent germination of clover mildew conidia on glass slides at 100 per cent relative humidity, 20° C. and in light for 8 hours*

| Period of exposure at 35° C. (minutes) | Conditions under which spores were exposed | | |
|---|---|---|---|
| | detached spores on dry slides | Spores attached to mycelium on which they were produced | |
| | | leaf pieces on dry glass plate | leaf pieces floating on water |
| | *Per cent* | *Per cent* | *Per cent* |
| 0 (check) | 33 | 29 | 49 |
| 5 | 47 | 21 | 56 |
| 20 | 80 | 58 | 51 |
| 80 | 0[a] | 0[a] | 72 |
| 320 | 0[b] | 0[b] | 32 |

[a] Spores not visibly shrunken.

[b] Spores shrunken.

pieces of leaves that can be easily dried out, they are soon killed, while, when attached to a living leaf kept alive on water, they are but slightly injured at this high temperature.

The effect of the host in protecting the conidia from the injurious action of combined low humidity and high temperature is very pronounced under field conditions. In studies of the diurnal cycle of the germinability of conidia of clover mildew at Madison, Wisconsin, in 1933 (4), glass slides were dusted with conidia from field plants and some of these slides were exposed to the field summer environment, including temperature, sunlight, and humidity. Conidia on such slides were all shrivelled and killed in as short a time as 3 hours during the hottest part of the day, but similar conidia dusted upon a living leaf in a similar environment gave rise to successful infection. Also, conidia attached to the conidiophores on infected leaves remain turgid for several days under the normal summer environment in the field and in the greenhouse. The action of the host in protecting the conidia from the injurious action of low humidity and high temperature under field conditions possibly is due to the lower temperature and higher humidity at the leaf surface.

### THE CHANGE IN VOLUME OF CONIDIA DURING GERMINATION

Germination of the spores of most fungi requires free moisture and the germination process is characterized by intake of water and increase in volume of the spore. That this general rule does not apply to the conidia of certain powdery mildews already has been indicated, and further data were secured by measuring the changes in volume of conidia during the germination process. Conidia of *Erysiphe polygoni* from clover, *E. polygoni* from cabbage, and *E. graminis* from barley were dusted onto slides or cover glasses, and the length and diameter of individual conidia were measured at the time of preparation of the spore mounts, and after several hours' exposure to known relative humidities. Germinated and nongerminated conidia were tabulated separately.

As examples of spores believed to germinate normally in free moisture, the following were used: The urediospores of *Uromyces fallens* (Desm.) Kern from clover leaves and the conidia of *Sclerotinia fructicola* (Wint.) Rehm, *Collectotrichum trifolii* S. M. Bain, and *Cicinnobolus cesatii* de Bary from agar cultures. The conidia of these 3 latter fungi have failed to germinate on dry glass slides but some germination of the urediospores of clover rust has resulted on apparently dry slides at 100 per cent relative humidity. As these spores were germinated in or on the surface of water, it was impracticable to follow the changes in size of the same spore during germination. Therefore, random spores in the hanging drops were

measured at the time of preparing the mount and after known incubation intervals.

Spore volume was calculated from the formulae given in table 6, which were supplied by M. A. Ingraham, Department of Mathematics, University of Wisconsin, on the basis of drawings supplied by the writer. Spore shape changed but slightly during germination, and the same formula for volume was used before and after germination.

The number of spores measured, germination interval, environmental conditions during germination and changes in volume of spores for each species tested, are given in table 6. The powdery mildew conidia decreased about 24 per cent in volume during germination, rust urediospores showed no change in size, and the conidia of the other fungi tested showed a pronounced increase in size during germination. All spores, whether germinated or not, remained turgid during the period of these tests. The volume changes of the nongerminated spores (not tabulated) was about the same as that of the germinated spores, *i.e.*, nongerminated conidia of Erysiphe decreased in volume, nongerminated rust spores remained the same size, and those of Colletotrichum, Sclerotinia, and Cicinnobolus increased in volume under these experimental conditions.

When conidia of *Erysiphe polygoni* from clover and from cabbage were floated on water or placed on dry slides at 100 per cent relative humidity, there was also a decrease in volume of the conidia during germination, but the decrease was not so large or so significant as at 80 per cent relative humidity.

THE EFFECT OF RELATIVE HUMIDITY ON THE GROWTH OF MYCELIUM OF CLOVER POWDERY MILDEW. BERKELEY, CALIFORNIA. 1935

Excised red clover leaves with their petioles in stoppered vials of water were inoculated and placed, after 4 hours in the laboratory, into preparation dishes containing water, or known concentrations of sulphuric acid. The preparation dishes were placed at known temperature for 24 hours or longer, and the total length of mycelium per mildew colony was determined from measurements of 5 mildew colonies on each of 3 leaflets in each humidity condition tested. According to the results of 4 tests (table 7) with young leaves from the same plant, the size of mildew colonies was less at 0 relative humidity than at 100 per cent relative humidity, but the differences were not great except in 2 tests at 25° C. and 27° C. in which no growth of mycelium occurred at 0 relative humidity. Relative humidities intermediate between 0 and 100 per cent also were tested, but significant differences due even to relatively large differences in relative humidity were observed in only 2 cases.

TABLE 6.—*Changes in size of spores during germination, Madison, Wis., 1933*

| Test fungus | Number of spores measured | Germination interval in hours | Substrate and environment | Average length of spores at beginning of germination interval | Average length of spores at end of germination interval | Average diameter of spores at beginning of germination interval | Average diameter of spores at end of germination interval | Formula used in calculation of spore volume[a] | Increase or decrease in spore volume during germination |
|---|---|---|---|---|---|---|---|---|---|
| | | | | μ | μ | μ | μ | | % |
| *Erysiphe polygoni* from clover | 30 | 10 | 17° C. 80% R. H. | 34.7 | 34.1 | 18.6 | 15.7 | $1/6\ \pi\ ld^2$ | − 30.0 |
| *E. polygoni* from cabbage | 15 | 24 | 20° C. 80% R. H. | 44.7 | 43.7 | 16.4 | 13.6 | $1/6\ \pi\ ld^2$ | − 32.8 |
| *E. graminis* from barley | 13 | 5 | 20° C. 80% R. H. | 33.1 | 32.3 | 12.1 | 11.7 | $1/6\ \pi\ ld^2$ | − 8.9 |
| *Uromyces fallens* from clover | 22 | 11 | 19° C. $H_2O$ | 23.6 | 23.6 | | | $1/6\ \pi\ d^3$ | 0 |
| *Colletotrichum trifolii* | 22 | 11 | 19° C. $H_2O$ | 14.9 | 16.4 | 3.1 | 3.9 | $.61\ ld^2$ | + 74.3 |
| *Sclerotina fructicola* | 45 | 6 | 19° C. $H_2O$ | 11.7 | 13.6 | 7.5 | 10.9 | $1/6\ \pi\ ld^2$ | + 146.0 |
| *Cicinnobolus cesatii* | 25 | 2 | 19° C. $H_2O$ | 8.5 | 9.0 | 2.7 | 3.3 | $.61\ ld^2$ | + 58.0 |

[a] $\pi = 3.1414$; l = length of spore; d = diameter of spore.

TABLE 7.—*The effect of relative humidity on the growth of mycelium of clover mildew as determined by four tests conducted at Berkeley, California, 1935*

| Date of test | Temperature at time of test | Time interval of test | Total length of hyphae per colony in microns | |
|---|---|---|---|---|
| | | | 100 per cent relative humidity | 0 relative humidity |
| April 12 | 22° C. | 26 hours | 399 | 210 |
| April 12 | 22° C. | 43 hours | 633 | 451 |
| May 4 | 27° C. | 24 hours | 352 | 0 |
| May 6 | 16° C. | 24 hours | 95 | 108 |
| May 6 | 25° C. | 24 hours | 370 | 0 |
| May 12 | 16° C. | 24 hours | 114 | 99 |
| May 12 | 25° C. | 24 hours | 325 | 248 |

## THE RELATION OF WEATHER TO MILDEW DEVELOPMENT

During the summers of 1930, 1931, and 1932, in Indiana, and 1933, in Wisconsin, the powdery mildew of red clover was prevalent but, apparently, developed most luxuriantly during periods of dry weather. The scarcity of clover mildew in the early part of the season is believed to have been due to the scarcity of inoculum, and not to the weather conditions prevailing, as artificial inoculations in the field in Lafayette, Indiana, in 1932, were successful a month before mildew was found occurring naturally. Clover mildew was always more severe late in the season and on the second crop. During September and October, 1930, 1931, 1932, and 1933, mildew was less severe than during July and August, due, in part it is believed, to the direct injurious effect of the fall rains on mildew development and to the extensive development of *Ciccinobolus cesatii*, a fungus parasite of powdery mildews, which requires free moisture for dissemination of its conidia.

In the region of San Francisco Bay, California, the abundance of powdery mildews and the relative scarcity of other fungous diseases of foliage during the virtually rainless, though somewhat foggy, summer months, is very pronounced. In the dry and relatively fogless summers of the Sacramento and San Joaquin valleys, powdery mildew of grapes is one of the most prevalent and serious (if uncontrolled) diseases. During the rainy season (winter) of the San Francisco Bay region, on the other hand, powdery mildews, with the notable exception of that of cereals, are very scarce, while downy mildews are relatively abundant.

Most clover-mildew inoculations in the field, even those made in the hottest driest hours of sunny days, have been successful; and in no case has failure to secure infection been shown traceable to low humidity,

although several cases of poor infection have resulted. To determine further the relation of weather, especially rain, to mildew development in the field, 2 areas of clover, 3x6 ft. in area, at Madison, Wisconsin, were covered on August 2, 1933, shortly after the first growth of clover had been removed by cutting, with glass sashes at 16 in. above the soil level. These sashes protected the plants from rain, but as no protection was offered on the sides, the humidity and other environmental conditions under the sashes should have been only slightly different from those in the open. The soil below the sashes became rather dry; but, while the protected plants did not make so much growth as did those in the open, they remained in good condition and did not wilt.

There was 4.87 inches of rain with 24 cloudy or partly cloudy days from August 2, when the sashes were put in place, to September 22, when the test was considered completed. On September 22, the mildew on the exposed plants was sparse, or was heavily infected with *Cicinnobolus cesatii*, or both. On the plants protected by the cold frames, the mildew was not parasitized by Cicinnobolus, and the vigorous mycelium was producing an abundance of conidia. The mildew development on the protected plants was about twice as severe as on the exposed plants.

Besides the effect of Cicinnobolus in controlling the mildew on the exposed plants, the rain, during the period, apparently directly inhibited mildew development. Apart from this test and the one reported later, dealing with conidial dissemination after a rain, the effect of rain on clover mildew in the field has not been tested. With excised clover leaflets on sugar solution, however, mildew was considerably checked or was killed out when inoculated leaflets were atomized with water for a few seconds daily, or only once within 2 days after inoculation. With powdery mildew of peas a single vigorous atomization with water for 15 seconds at 2 or 4 days after inoculation completely washed the mycelium from the leaves and it was not regenerated from the haustoria that remained in the epidermal cells. On mildewed mustard and bean plants exposed to about 2 inches of rain from 3 p. m., February 28, to 8 a. m., March 1, 1935, at Berkeley, California, all the conidiophores were destroyed and much of the mycelium was washed away on the upper surfaces of the leaves, while check plants under a bell jar had a luxuriant development of conidiophores and conidia.

The low soil moisture in the covered area of the above mentioned clover plot probably had little, if any, effect on the mildew development. In duplicated tests in soil cans in the greenhouse, clover mildew developed very slightly better on clover plants grown in soil held at 40 per cent of its water-holding capacity by additions of water to the surface of the soil than

to soil at 20, 60, 80, or 100 per cent of its water-holding capacity. With beans there was no marked difference in the development of powdery mildew on plants growing in soil at 25, 50, 75, and 100 per cent of its water-holding capacity.

Rain may markedly check the normal formation and dissemination of conidia, as would be expected from the injurious action of rain on the conidiophores. A total of 0.32 in. of rain fell from 1:00 p. m. to 2:05 p. m. on July 14, 1933, at Madison, Wisconsin; then there was no rain for several days, and the weather was clear and bright. Exposure of clean glass slides as spore traps in 3 locations in a field of heavily mildewed plants was started at 7 p. m., July 14. The results, based on counts of the conidia on 2.5 sq. cm. on each of the 3 slides, are given in table 8. No condidia were caught until the interval from 11:45 p. m., July 14, to 8 a. m., July 15, when only 2 were caught. The following day (July 15) conidial dis-

TABLE 8.—*Conidial dissemination of Erysiphe polygoni in a clover field after a rain at Madison, Wisconsin, 1933*

| Date | Period | Weather | Total number of spores per $cm^2$ per hr. | Average number of spores per $cm^2$ per hr. |
|---|---|---|---|---|
| July 14 | 1:08– 2:05 p.m. | .32″ rain | | |
| | 7:00– 8:30 p.m. | cool, cloudy | 0 | 0 |
| | 8:30–11:45 p.m. | " " | 0 | 0 |
| July 14–15 | 11:45 p.m.–8:00 a.m. | " " | 2 | .03 |
| July 15 | 8:00 a.m.–2:00 p.m. | bright | 554 | 12.1 |
| | 2–4:30 p.m. | " | 382 | 20.2 |
| | 4:30 p.m.–7 p.m. | " | 258 | 13.8 |
| July 15–16 | 7:00 p.m.–10:00 a.m. | " | 183 | 1.6 |
| July 16 | 10:00 a.m.–1:30 p.m. | " | 498[a] | 47.4 |
| | 1:30 p.m.–3:30 p.m. | " | 400[b] | 44.4 |
| | 3:30–6:00 p.m. | " | 286[b] | 25.4 |

[a] Spores counted only on 1 $cm^2$ on each slide.

[b] Spores counted only on 1.5 $cm^2$ on each slide.

semination was greatly increased but was still only about half that of the third day. Similar results have been secured when mildewed leaves of clover, bean, and mustard were exposed to rain in Berkeley, California, during March, 1935.

High humidity reduces the number of conidia formed. In 15 out of 19 tests on potted plants, or excised leaves with their petioles in water, there was a greater number (300 per cent greater on the average for the 19 tests) of conidia liberated from mildewed clover leaves held at 24 to 49

per cent relative humidity than from leaves held at 94 to 100 per cent relative humidity in laboratory and greenhouse tests at Madison. At Berkeley, similar results were secured with mustard mildew and bean mildew. In the tests at Madison, only conidia that were passively liberated by the mildewed leaves were caught on the spore-trap slides, while in the tests at Berkeley, the test leaves were shaken over spore-trap slides at the bottom of a cylinder. The results, being similar in both types of test, indicate that the cause of the fewer conidia caught from the leaves at high humidity in the Madison tests was not entirely due to their failure to abscise from the conidiophores in the humid atmosphere.

Inoculations performed by dusting conidia onto dry leaves of disease-free plants in the afternoon of clear, sunny days, with no provision for conditions of incubation, have been successful with clover mildew in the field at Lafayette, Indiana, and Madison, Wisconsin, and with sunflower, mustard, peach, and cucumber mildews in the normal outdoor summer environment at Berkeley, California. Similar inoculations were successful in the greenhouse with clover, barley, and cabbage mildews at Madison, and with sunflower, mustard, cucumber, cantaloupe, dandelion, clover, knotweed, bean, barley, oats, and pea mildews at Berkeley. In the laboratory at Berkeley, with relative humidity varying between 25 and 55 per cent, but generally about 44 per cent, mildew infection was successful on excised leaves of rose, sunflower, mustard, cucumber, cantaloupe, clover, and bean. Potted plants of red clover, bean, sunflower, and cantaloupe, inoculated February 16, 1935, with their respective mildews, were placed in chambers with light (about 900 foot candles and a 16-hour day) temperature (25° C.), and 50 per cent relative humidity controlled, and with rapid, forced-air circulation over the plants. All developed a luxuriant growth of mildew, except sunflower, on which it was sparse. In no case has failure to secure infection with any of the powdery mildews studied been traced to low relative humidity.

## DISCUSSION

The tolerance of *Erysiphe polygoni* to low humidity as considered in this paper, accords with the diurnal cycle of *Erysiphe polygini*, previously described. Natural inoculation with *E. polygoni* probably occurs in greatest abundance during the daylight hours of normal sunny days, and infection (the establishment of nutritional relations between host and parasite) probably occurs a few hours later. Free moisture or high humidity are not necessary or desirable at any stage in the conidial life history of this parasite, though very low humidity may be injurious under certain conditions, especially at high temperature. This adaptation to environment should enable this fungus to become a virulent parasite during

the summer season of many climes. That *E. polygoni* is not more abundant than it is may be due to a number of factors, most of which are only imperfectly understood.

Under field conditions, low relative humidity and high temperature are frequently coincident. While *Erysiphe polygoni* is very tolerant to low humidity at low temperature, the injurious action of low relative humidity at high temperature is pronounced. Under laboratory conditions at 100 per cent relative humidity, the maximum temperature for mildew infection of mustard and sunflower was 28° to 31° C., and, for that of clover, pea, and bean, it was between 31° and 34° C., at Berkeley. As temperatures approaching and exceeding these are not uncommon where these crops are grown and these diseases are sometimes prevalent, the caustive fungi can likely tolerate them for short periods (Table 6), but it is probable that at these high temperatures dry weather is injurious to them. Most of the writer's data showing the injurious action of low relative humidity were secured where the conidia were on dry glass slides. In nature, on host leaves, the injury from low humidity would be much less.

Though, under certain conditions, better development of *Erysiphe polygoni* has resulted under conditions of low than under those of high humidity, the writer believes that high humidity is not in itself, markedly injurious to the powdery mildews studied. The luxuriant development of the powdery mildews of clover, pea, bean, rose, dandelion, knotweed, cucumber, cantaloupe, sunflower, and lilac on excised leaves floating on sucrose solution in closed Petri dishes, would preclude this idea. Rather, the writer believes that the forms studied can develop luxuriantly over a wide range of relative humidities and that they are especially well adapted, in contrast with most parasitic fungi, to very dry atmospheric conditions.

The writer does not disagree with the numerous observations by others that certain powdery mildews, including strains of *Erysiphe polygoni,* are frequently more severe in shade or in various types of specially protected environments, but he believes that except where we are concerned with a primary infection center, such as overwintered mycelium, or perithecia, it is generally some factor other than the effect of relative humidity on the fungus that is mainly responsible for this local severity of mildew. The lower temperatures of the air, the greater vigor and succulence of the plants, and the thinner cuticle of the leaves may favor mildew development in some cases. There probably is, also, great variation in different species and strains of the powdery-mildew fungi. The writer's results with *E. polygoni* from mustard, *E. graminis,* fram barley, *E. cichoracearum* from sunflower, and *Sphaerotheca pannosa* from rose, indicate that these fungi are considerably less tolerant to low relative humidity than the strains of *E. polygoni* from clover and bean.

SUMMARY

At favorable temperatures, conidia of *Erysiphe polygoni* from red clover and from bean have germinated well at relative humidities ranging from 100 per cent to approximately 0. In some cases more germination resulted at approximately 0 relative humidity or after several hours drying, than at 100 per cent relative humidity, while in others the highest germination occurred at high relative humidity. Even at temperatures favorable for germination, the germinated and nongerminated conidia were soon shrivelled and killed at low relative humidity.

Conidia attached to conidiophores on living mildewed leaves or detached conidia on the surface of healthy leaves were less injured by low relative humidity than conidia on detached mildewed leaves, allowed to dry out, or than detached conidia on dry glass slides. The injurious action of low humidity was increased at high temperatures,

Conidia of *Erysiphe polygoni* and *E. graminis* decreased considerably in volume, but remained turgid at 80 per cent relative humidity. Conidia at 100 per cent relative humidity or floating on water showed a less significant decrease in volume during germination. In contrast conidia of *Colletotrichum trifolii, Sclerotinia fructicola,* and *Cicinnobolus cesatii* did not germinate without free water, and increased greatly in volume during germination.

Mycelium of clover mildew grew well at relative humidities ranging from 0 to 100 per cent at low temperatures. At high temperatures and low relative humidity, the mildew colonies were killed in some tests.

Different levels of soil moisture were found to have no definite effect on the development of clover and bean mildew.

Infection with the powdery mildews of clover, bean, cabbage, barley, cantaloupe, delphinium, and mustard has resulted from inoculation made at the lowest relative humidities tested.

Conidiophores of bean and mustard mildew were destroyed during a heavy rain; mildew on peas or beans were injured or destroyed by atomizing the infected leaves with water, and the dissemination of the conidia of clover, bean, and mustard mildew was severely checked by rain. Clover mildew developed better in the field when protected from rain.

General field observations also indicate that *Erysiphe polygoni* and certain other powdery mildews are adversely affected by rain and can develop luxuriantly and possibly better under rather dry conditions.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA.

# IMMUNITY OF VIKING RED CURRANT FROM WHITE PINE BLISTER RUST UNDER FIELD CONDITIONS

GLENN GARDNER HAHN[1]

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## INTRODUCTION

As an integral part of the investigation concerned with the resistance of Viking (Syn.: Rød Hollandsk Druerips), a Norwegian red-currant variety, to white pine blister rust (*Cronartium ribicola* Fischer), extensive field testing was carried on. These tests in the open, supplemented rigorous artificial experiments in the greenhouse (2, 3),[2] conducted in 1929 to 1932, in which the introduced variety, cultivated for many years in Norway because of its productiveness, was found to be highly resistant to British and North American strains of blister rust. Moreover, greenhouse results have corroborated field experience with the variety in Norway, where, over a period of many years, Norwegian horticulturists and pathologists have not observed *C. ribicola* fruiting on the leaves of the Viking currant. In Norway, blister rust has been most destructive (4, p. 74–82; 5), and *Pinus strobus* L., extensively planted there, must be considered a dying species.

Taking into account the resistance of Viking to Norwegian strains of blister rust under natural conditions, and its high resistance to British and North American strains under artificial conditions, it was not to be expected that environmental conditions in this country would fundamentally change the physiologic processes of the Norwegian variety to the extent of breaking down resistance to rust. If such an alteration took place, explanation of this phenomenon could be sought in the fact that there were biologic forms of *Cronartium ribicola,* some more pathogenic than others.

To determine this point, Viking was planted widely in the East and West for field testing against strains of blister rust occurring in different parts of the white-pine regions. Optimum conditions for natural infection were obtained in New York, Connecticut, Massachusetts, New Hampshire, Maine, Oregon, and Canada (Ontario). Through the able cooperation of J. F. Martin, E. C. Filler, and other members[3] of the Division of Plant

[1] Conducted as a cooperative project between the Bureau of Plant Industry, the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, and the Osborn Botanical Laboratory, Yale University.

[2] Reference is made by number to Literature Cited, p. 875.

[3] For their splendid and indispensable cooperation in this experiment, the writer is particularly indebted to the following members of the Division of Plant Disease Control: Connecticut, Mr. J. E. Riley; Maine, Messrs. W. O. Frost, D. S. Curtis, G. H. Kimball, J.

Disease Control, Bureau of Entomology and Plant Quarantine, these field experiments were extended over a period of 3 years, 1932 to 1934. Various planting sites for this experiment were provided, the purpose being to select diverse environmental types in order to study the vegetative development of the Viking and its reaction to disease over a wide range of field conditions in North America.

## FIELD EXPERIMENTS WITH WHITE-PINE BLISTER RUST

### Plant Material for the Field Test

To test the resistance of Viking under natural conditions, young plants were propagated from cuttings at the pathological greenhouses, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C. The cutting stock was obtained in 1930 from Prof. Olav Moen, Norwegian Agricultural High School, Ås, Norway. The young plants were maintained under conditions free of the Japanese beetle at Washington, D. C., and later at the field station of the Division of Plant Exploration and Introduction at Glenn Dale, Maryland, during the winter of 1931–1932. In April, 1932, these 2-year old plants, uniformly thrifty and more or less dormant, were shipped under special permit.[4] They were planted on selected sites previously prepared for the field experiments.

### Planting Sites

Planting sites were selected by members of the Division of Plant Disease Control, due attention being paid to the temporary establishment of Viking in an environment where prevailing winds would be certain to carry abundant aeciospores of white pine blister rust during the spring, to its leaves.[5]

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M. White; Massachusetts, Messrs. C. C. Perry, E. M. Brockway, William Clave, G. S. Doore, R. E. Wheeler, K. K. Stimson; New Hampshire, Messrs. L. E. Newman, F. J. Baker, S. H. Boomer, T. L. Kane, T. J. King, L. C. Swain; New York, Messrs. P. E. Barber, B. H. Nichols, H. G. Woodward. The above-mentioned collaborators were responsible for the selection of planting sites, maintenance of the test plots and all the records on the experiment. Mention should be made here of valuable assistance also rendered by Mr. George B. Dorr, Superintendent, Acadia National Park, Mr. C. E. Dow, Dr. A. E. Brower, Entomologist, Maine Forest Service, and Mr. W. I. Leland, Technical Foreman, McFarland C. C. C. Camp, all of Bar Harbor, Maine; by Dr. H. T. Güssow, Dominion Botanist, Central Experimental Farm, Ottawa, Canada; and by Mr. L. N. Goodding, formerly of the Division of Plant Disease Control, Corvallis, Oregon, now of Soil Conservation Service, and Mr. H. G. Lachmund, formerly of the Division of Forest Pathology.

[4] Acknowledgment is made of the helpful cooperation of Mr. H. A. Gunning in caring for and preparing the Viking plants for shipment.

[5] The following cooperators provided planting sites for the Viking field tests: Connecticut, Messrs. E. C. White, Litchfield, W. E. Swift, Cornwall, and D. E. Harris, Salisbury; Massachusetts, Messrs. G. B. Caswell, Holden, E. T. Wilson, East Princeton, Stephen Taylor, Supt., and G. F. Burgess of the New Bedford Water Works, H. L. Bates,

The sites generally were set about with natural pine infection on all sides, the distance from the source of aeciospores varying from 15 to 1500 feet, commonly, 30 to 300 feet.

The following soil types were chosen for planting:

(a) Rich garden soil consisting of a well-drained, sandy loam kept in tillage by the cultivation of other small fruit or vegetable crops (Fig. 1, A).

(b) Heavy clay garden soil lacking fertilization and containing admixtures of peat.

(c) Old pastures or abandoned fields needing fertilization; the soil light and gravelly and competition with weeds and grasses particularly heavy.

(d) Old pastures where the soil was heavy with considerable clay (Fig. 1, B).

(e) Dry, sandy loam in artificial clearings made in dense mixed second growth stands of young white pine and broad-leaf species.

Where fertilization was needed, well-rotted manure was put upon the test plots. Irrigation also was provided wherever possible. The first summer (1932) of the field tests was particularly dry, and drought conditions prevailed quite generally throughout New England. As a consequence, the Viking plants set out in woodland areas suffered from drought injury. Test plants, however, situated on accessible garden sites where water could be applied, were not retarded and made excellent seasonal growth. Garden sites were exposed as rigorously to rust infection as those situated in outlying wooded areas, where, in the main, natural precipitation had to be relied upon.

## Inoculum

As has been stated above, the Viking was tested in close proximity to cankered pine, which served as a source of ample aeciospore inoculum in the spring. In one instance (Lewis, N. Y.) some of the currants were planted beneath young diseased white pines, so that fertile infection, if such were possible, could hardly be avoided. In certain instances the test plants were dusted artificially with aeciospores at a time when atmospheric conditions were favorable for infection.

During the field testing, 1932 to 1934, abundant aeciospore inoculum was available. Despite the drought condition of 1932, local infection was evident on Ribes in New England, particularly on the wild or pasture goose-

Worthington, H. C. Chapin, Sheffield; Maine, Messrs. G. B. Dorr, Bar Harbor, D. C. Osborn, Camden, G. W. Grover, Freeport, F. Barry and W. D. Whitten, N. Augusta, Mrs. Gertrude Leighton, Bridgton; New Hampshire, Messrs. L. C. Swain, Brentwood, Ernest Hunter, Tuftonboro, Irving Wilson, New Boston, T. B. Southwick, E. Acworth, Paul Dickinson, Lisbon; New York, Messrs. P. E. Barber, Hadley and Corinth, N. H. Harpp, Chestertown, J. E. Crossett, Lewis, Albert Dashnaw, Peru. Acknowledgment is made of this generous cooperation.

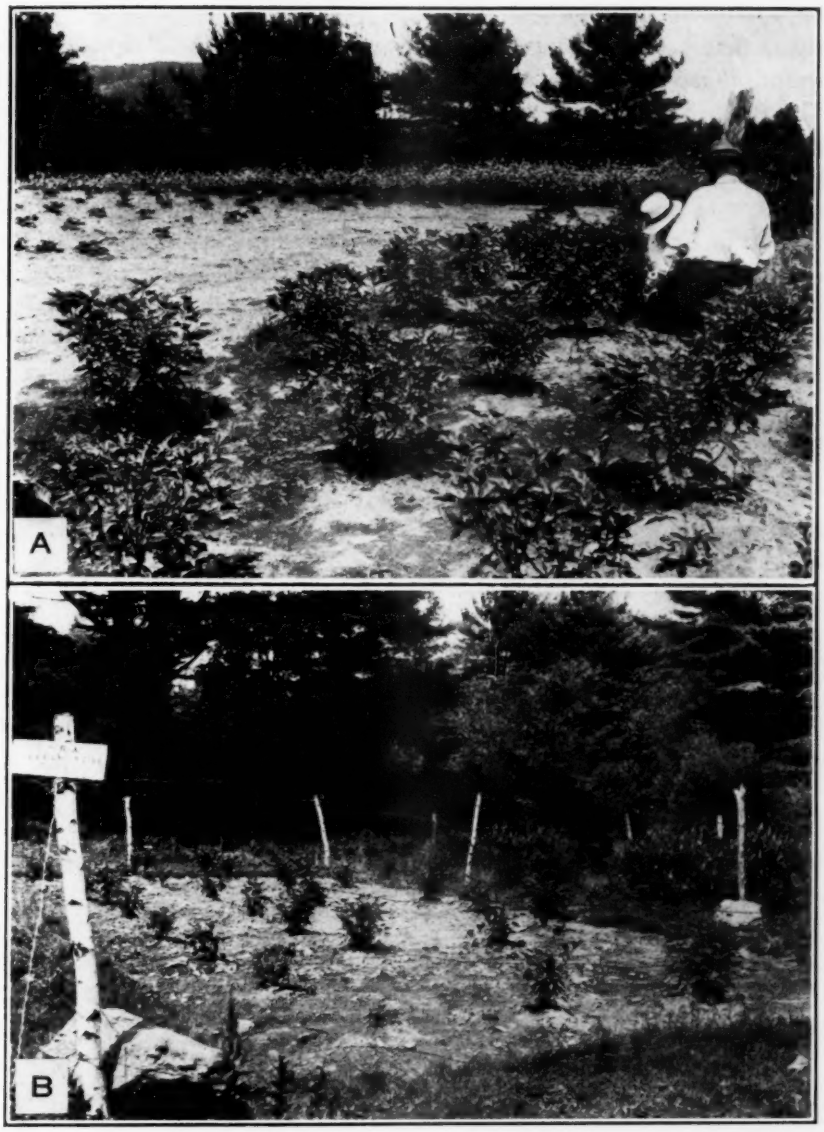


FIG. 1. Test plots surrounded by blister-rust-infected pine (*Pinus strobus*), which provided abundant aeciospore inoculum for natural infection of Viking. A. Plot at Chestertown, N. Y. Viking growing on good garden soil. Planted susceptible Ribes serving as checks on the presence of rust inoculum, became infected with *Cronartium ribicola*. B. Plot at Sheffield, Mass. Viking growing in an old pasture on heavy soil. Abundant natural infection of blister rust was observed on *Grossularia cynosbati* and cultivated red currants growing in the vicinity. A, photographed July 30; B, Aug. 1, 1934.

berry, *Grossularia cynosbati* (L.) Mill. In Massachusetts[6] infection was heavier that year than was expected, and in Maine[7] the American black currant, *Ribes americanum* Mill., showed more than the average amount of infection. That same year black currants, *R. nigrum* L., were also heavily infected in Massachusetts. Abundant aeciospore inoculum likewise was available in 1933 and 1934. The last year of testing, more fruiting cankers were reported in northern New Hampshire[8] than in any previous year and in New York[9] blister rust lesions, many of which were large with an abundance of spores, were particularly conspicuous. Urediospore inoculum was also available for infection of the Viking, on the leaves of susceptible Ribes either growing naturally about the test plots or planted beside the Viking when the test plants were set out.

## Checks on the Field Test

In order to be doubly certain that Ribes plants were becoming infected in the immediate neighborhood of the test plants, susceptible "check" plants were planted along with the Viking. In most cases native species such as *Grossularia cynsobati* and *Ribes glandulosum* Grauer (skunk currant) were used; in one instance the very susceptible black currant was utilized. It was the general procedure to remove the infected leaves of the check plants when telia appeared, in order that the rust would not continue to spread to pine in the neighborhood of the plot.

Where susceptible Ribes checks were not set out in the experimental plots, statements could be readily substantiated that natural inoculum was available by checking on infection on nearby wild Ribes, or on cultivated or escaped red garden currants [*R. sativum* (Reichenb.) Syme (*R. vulgare* Auct. not Lam.)] growing near the test plots. An additional guarantee that ample inoculum was present for infection was provided in certain instances where artificial inoculations in the field were performed. These artificial tests were made despite the fact that it was practically certain that aeciospore inoculum was being distributed, naturally, in the vicinity of the test plants. This additional evidence was sought by the field men to substantiate fully the claim that Viking was immune.

## Observations and Records

Records on infection of the Viking (each plant bore a metal tag with an assigned number), together with those for the associated susceptible

[6] C. C. Perry, in, U. S. Dept. Agr. Plant Indus., Blister Rust News 16: 129, 156, 165. 1932 [Mimeographed].

[7] J. M. White, in, *Ibid.*, 16: 168. 1932 [Mimeographed].

[8] T. L. Kane, in, *Ibid.*, 18: 92. 1934 [Mimeographed].

[9] H. Holcomb, in, *Ibid.*, 18: 95. 1934 [Mimeographed].

check Ribes, were made every fortnight beginning May 15 and ending September 15. Leaves were examined carefully for fertile infection (uredia and telia) and necrotic flecks (2) recorded where fertile infection did not occur.

A record was also kept of the vegetative growth made by the introduced Norwegian variety on the various sites. Measurements were made, May 15 and September 15, of the total length of live stem, living canes and branches per bush, in inches for the year. An average was calculated for the plot, not only for the total length, but, also, for the increase in live stem in inches for the year.

## RESULTS OF FIELD TESTS, 1932 TO 1934

### In New England and New York

During the period of 3 years' field testing, 927 plants of the introduced Viking were highly resistant to white pine blister rust. They did not produce fertile infection (uredia or telia), although they were exposed to strains of *Cronartium ribicola* in blister-rust areas in New England and New York, where the disease of white pine has been very severe and destructive. As stated above, ample aeciospore inoculum was available during the field experiment, which involved a total of 25 test plots, so that the Viking was thoroughly subjected to natural infection. Susceptible Ribes, set out as check plants, readily became infected. Where Ribes checks were not planted, natural infection, resulting from rust spores distributed over the test plots, could be ascertained by the presence of rust, fruiting on the leaves of wild or cultivated Ribes growing in the vicinity of the Viking test plots. Seven of the total number of test plots wkere planted near cultivated garden currants, which became naturally infected with the rust, *i.e.*, currants near the test plot located on a rich garden site at Worthington, Massachusetts. Here the Viking grew vigorously, its luxuriant foliage entirely free of uredia or telia, whereas the leaves of red-currant plants growing in close proximity were heavily infected with rust.

As an additional check on the presence of ample inoculum and natural infection, necrotic flecks were observed in many instances on the leaves of the highly resistant Viking. These hypersensitive flecks, as previously described (2), consisted of necrotic leaf cells surrounded by hypertrophied spongy mesophyll and palisade tissue lacking chlorophyll. These flecks were produced only on tender, very young, expanding or recently expanded leaves. They never were observed to produced fertile fruiting. Fully mature leaves of Viking showed no presence, whatsoever, of either necrotic flecks or fertile fruit bodies. In table 1, a summary of results obtained in the field tests is presented.

TABLE 1.—*Summary of data on Viking field tests in New England and New York,*[a] *1932 to 1934*

| Location of plots | Viking test plants | | | | Source of aeciospores *Cronartium ribicola*[c] (distance ft.) | Infection data for 3-year period | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Number of bushes | | Vegetative growth 1934 | | | Viking | | Susceptible Ribes checks | |
| | Planted 1932 | Alive[b] 1934 | Av. amount of live stem per bush (inches) | Av. increase in live stem per bush (inches) | | Type of Reaction | Necrotic flecks | Species[d] | Infection degree |
| *Maine* | | | | | | | | | |
| Bar Harbor | | | | | | | | | |
| Plot I | 131 | 129 | 427.6 | 283.6 | 25 | Immune | Positive | (8) *Ribes glandulosum*<br>(1) *R. sativum* hybrid | Abundant |
| | | | | | | | | American Red Dutch | Do |
| Plot II | 65 | 65 | 595.3 | 302.3 | 15 | Do | Do | (3) *R. glandulosum* | Do |
| | | | | | | | | (1) American Red Dutch | Do |
| Camden | 20 | 20 | 879.6 | 683.6 | 30 | Do | Do | *R. sativum* hybrid | Moderate |
| Freeport | 40 | 40 | 1004.7 | 768.7 | 30 to 300 | Do | Do | *Grossularia reclinata* | .....[e] |
| North Augusta | | | | | | | | | |
| Whitten Plot | 25 | 24 | 69.5 | 24.5 | 300 | Do | Do | ..... | ..... |
| Barry Plot | 15 | 15 | 115.9 | 76.9 | 300 | Do | Do | ..... | ..... |
| Bridgton | 40 | 39 | 149.8 | 59.8 | 75 | Do | Do | *R. glandulosum* | Abundant |
| | | | | | | | | *G. cynosbati* | Do |
| *New Hampshire* | | | | | | | | | |
| New Boston | 40 | 40 | 298.9 | 207.9 | Surrounded; heaviest ⅓ mi. due west | Do | ..... | ..... | ..... |
| Lisbon | 40 | 36 | 84.9 | 36.9 | 400 | Do | ..... | ..... | ..... |
| E. Acworth | 40 | 38 | 75.2 | 27.2 | 45 | Do | ..... | *G. cynosbati* | Abundant |
| Tuftonboro | 40 | 37 | 357.7 | 255.7 | 250 | Do | | Do | Do |
| Brentwood | 40 | 40 | 69.4 | 32.4 | Surrounded | Do | ..... | ..... | ..... |
| *New York* | | | | | | | | | |
| Peru | 36 | 32 | 52.3 | 9.3 | 200 | Do | Positive | (4) *R. sativum* hybrid | Scant |
| Lewis | 40 | 38 | 88.8 | 11.3 | 16 | Do | Do | Do | Moderate to |
| Chestertown | 39 | 37 | 208.0 | 86.0 | Vicinity | Do | Do | (1) Do | abundant |
| | | | | | | | | (1) *G. reclinata* | Do |
| Hadley } | 40 | 36 | 39.4 | 22.4 | Do | Do | ..... | (1) *R. nigrum* | Do |
| Corinth }[f] | | | | | | | | (1) *R. sativum* hybrid | Do |
| | | | | | | | | (1) *G. cynosbati* | Do |

TABLE 1.—(*Continued*)

| Location of plots | Viking test plants | | | | Source of aeciospores *Cronartium ribicola*[c] (distance ft.) | Infection data for 3-year period | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Number of bushes | | Vegetative growth 1934 | | | Viking | | Susceptible Ribes checks | |
| | Planted 1932 | Alive[b] 1934 | Av. amount of live stem per bush (inches) | Av. increase in live stem per bush (inches) | | Type of Reaction | Necrotic flecks | Species[d] | Infection degree |
| *Massachusetts* | | | | | | | | | |
| Worthington | 40 | 40 | 199.2 | 126.2 | 50 | Do | ..... | *R. sativum* hybrid<br>*G. cynosbati* | Moderate to abundant<br>Do |
| Sheffield | 40 | 32 | 113.1 | 69.6 | 30 | Do | Positive | Do<br>*R. sativum* hybrid | Do<br>Do |
| E. Princeton | 40 | 39 | 47.7 | 4.7 | 50 | Do | ..... | ..... | ..... |
| Holden | 40 | 39 | 95.3 | 42.3 | 1500 | Do | ..... | *R. sativum* hybrid | Moderate to |
| Lakeville | 40 | 40 | 69.5 | 14.5 | 100 | Do | ..... | *R. americanum* | Abundant |
| *Connecticut* | | | | | | | | | |
| Cornwall | 9 | 9 | 290.7 | 175.2 | Vicinity | Do | Positive | (1) *R. sativum* hybrid | Scant |
| Salisbury | 17 | 17 | 164.9 | 98.4 | 300 | Do | Do | ..... | ..... |
| Litchfield | 10 | 10 | 114.0 | 46.5 | 300 | Do | Do | ..... | ..... |
| Total: 25 Plots | 927 | 892 | | | | No production of uredia or telia | | Generally moderate to abundant infection white pine blister rust | |

[a] This table is based upon data collected by the cooperating white-pine-blister-rust agents, and compiled by Clave and Stimson under the direction of Filler.

[b] Certain of the Viking bushes were destroyed by vandals or killed by drought.

[c] Aeciospore inoculum was generally abundant throughout the field test.

[d] Numbers in parentheses before Ribes species indicate number of plants actually planted as checks in test plot. Unnumbered Ribes species represent plants already present in the vicinity of the plot when the Viking was set out for testing.

[e] Absence of data.

[f] Results on the 2 plots at Hadley and Corinth are combined. The checks are those planted in the Hadley plot where 10 Viking plants were set out.

In one instance, the very susceptible *Ribes nigrum* (1), was used as a check plant in the test conducted at Hadley, N. Y. This particular area abounds in cankered white pine, so natural infection of susceptible Ribes was to be expected. Such was the case, for in 1932, P. E. Barber stated (written communciation) that a black currant, the most congenial host for blister rust, planted beside the Viking, became so heavily infected that it was found necessary to destroy the plant; the Viking leaves, on the other hand, were devoid of rust fructifications. The following year (1933) Barber repeated this experiment, using as an additional check plant, a susceptible red garden currant. He artificially inoculated both the Viking and the checks with aeciospores of white-pine bluster rust. The leaves of the *R. nigrum* were reported (written communication) as being as heavily infected as in 1932, and began dropping by the first of August. The red-currant check plant also was infected. The Viking, however, maintained its resistance. This same reaction to rust on the part of the Norwegian variety was again evident in 1934. For the third time the Viking was inoculated with abundant aeciospore inoculum along with the susceptible checks consisting of wild gooseberry, a red currant and a small *R. nigrum* bush. Only the checks became infected.

Similarly, at Lewis, N. Y., in 1933, J. E. Crossett (verbal communication) upon whose farm the Viking had been planted for testing near and beneath cankered young white-pine trees, attempted to infect the Viking artificially by dusting the bushes heavily with aeciospores. He was wholly unsuccessful in obtaining infection on Viking.

The two test plots at Bar Harbor, Maine, (Table 1) were thoroughly exposed to blister rust, the 196 plants of Viking set out there being closely surrounded by diseased pine bearing vigorously fruiting cankers in the spring. The smaller plot was only 15 feet from heavily cankered white pine. In this area, natural infection on the susceptible Ribes checks took place readily. The first year of testing, K. K. Stimson artificially inoculated the Viking and one of two American Red Dutch, *Ribes sativum* (*vulgare*) hybrid check plants. These susceptible red-currant plants had been propagated from cutting material received from Norway in 1928, and were fully susceptible to blister rust in artificial inoculation tests (2). Stimson reported (written communication) that both American Red Dutch plants developed heavy infection, although only one of them was artificially inoculated, indicating that natural infection had proceeded as vigorously as artificial infection. Necrotic flecks were found on leaves of the Viking but no telia or uredia.

In figure 2, the Viking currant (Fig. 2, A) is shown in contrast with the American Red Dutch check plant (Fig. 2, B) at Bar Harbor. As stated

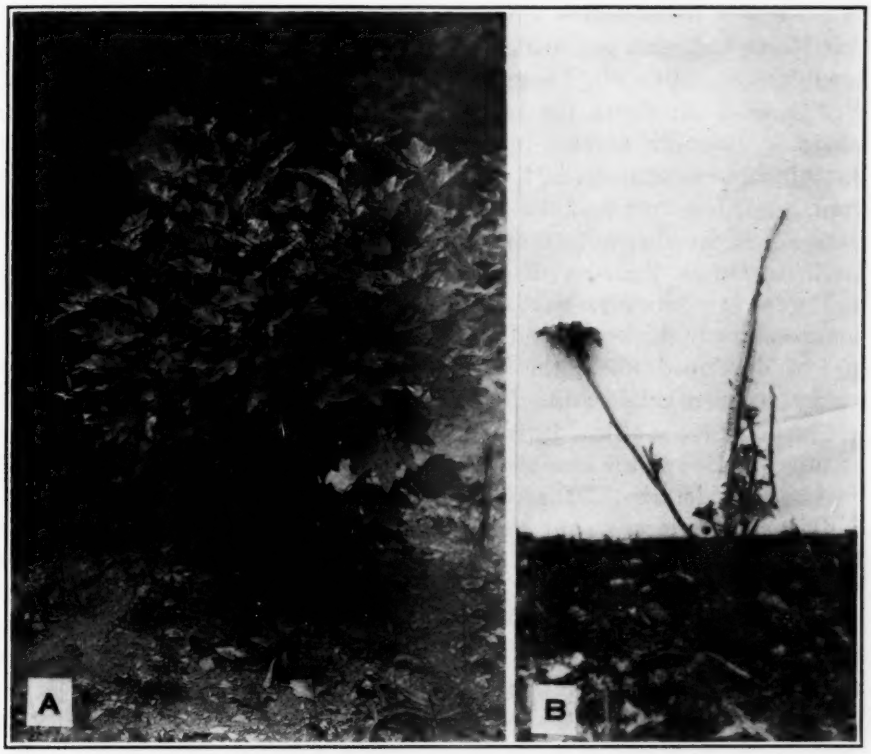


FIG. 2. Highly resistant Viking and susceptible red-currant check growing in Plot II, Bar Harbor, Maine, on the edge of a woodland on heavy clay soil. The plot was surrounded by white pine infected with blister rust. A. Fertile infection of *Cronartium ribicola* was not produced on the luxuriant foliage of Viking. Photographed July 19, 1934. B. Susceptible *Ribes sativum* hybrid var. American Red Dutch, planted as a check beside Viking. This plant became heavily infected each year during the field testing. Continuous stripping (see text) of the leaves bearing telia of *Cronartium ribicola* so reduced the vigor of the plant, that it was mostly dead. The photograph of the plant in flower was made May 21, 1935, on a scale slightly greater than that for A.

above, the leaves of the susceptible check Ribes, which became infected with the rust, were removed so that infection to nearby white pine would not continue to spread. Had the Red Dutch plants not been stripped of their infected leaves each year, growth of this susceptible variety would have approximated that of the resistant Viking. Continuous stripping by hand to remove leaves with telia, greatly weakened the check plant so that only a small part of its reduced growth was still alive. It is of course to be admitted, that a growth comparison in figure 2, A and B, is not altogether a fair one. In presenting this figure, it is the author's purpose to convey

to the reader the idea that natural infection of Ribes was taking place in this Maine test plot, and that the Viking, subjected to the same rigorous conditions for infection, was rust-free.

Figure 2, A, shows the luxuriant foliage produced at Bar Harbor, where, in 1934, the average increase in live stem per bush was 283.6 and 302.3 inches respectively, in the two test plots (Table 1). This vegetative growth was less than half that produced at Camden and Freeport, Maine, on sites where soil conditions were more favorable. The test plants at Bar Harbor were on the edge of a woodland containing diseased white pine and were, accordingly, partially shaded, so that leaf tissues did not become coarsely thickened, and the dark coppery-green, glossy, rugose type of leaf developed where the Viking was growing in the open on good garden soil, was not formed. Because of the diverse sites chosen for the 25 test plots, all types of leaf texture, that produced in shade, in partial shade, or fully exposed to the sun, were available for field testing.

In a consideration of the average amount of live stem per bush in 1934 and the average increase of vegetative growth for that year (Table 1), it can be readily seen that best growth was obtained in Maine, where the effect of the sea was felt. However, farther south and inland, where the sites were suitable for currants and where good culture prevailed, growth was correspondingly favorable, *i.e.*, Tuftonboro and New Boston, N. H., Worthington, Mass., and Cornwall, Conn. On such sites as those encountered on the Whitten plot, North Augusta, and the one at Bridgton, Maine, or those at Brentwood, N. H., Peru, N. Y., E. Princeton and Lakeville, Mass., where the Viking had been planted in dry, light, gravelly soil with an abundant weed competition, in old pastures or fields, or in woodland clearings, the Norwegian variety showed that it could still keep alive and maintain itself despite the adverse conditions of drought and water shortage encountered during the 3 years of testing. Given proper cultivation on favorable sites, the Viking should grow and fruit successfully over a considerable range of conditions.

### In Oregon

Forty plants of the Viking were introduced into Oregon for testing; 10 of them were turned over to G. M. Darrow, Division of Fruit and Vegetable Crops and Diseases, to be planted on the college farm of the State College of Agriculture east of Corvallis, and the remaining 30 taken to a Ribes garden established by L. N. Gooding above Rhododendron, Oreg., in the midst of diseased western white pine, *Pinus monticola* Dougl.

In the Rhododendron area, the Viking currant maintained its immunity from western strains of white-pine blister rust. Artificial inoculation experiments with eastern strains of *Cronartium ribicola* taken from *Pinus mon-*

*ticola* were performed in 1932 in the Rhododendron test plot by J. M. Mielke of the Division of Forest Pathology. While other Ribes species and varieties in the garden, which served as check plants, showed heavy infection, the Viking was resistant despite heavy artificial inoculation. In 1934 H. G. Lachmund reported (written communication) that, while all other Ribes species had been well infected, no trace of rust fruiting had been found on the Viking and that the plants had become well established and were making good growth. At Corvallis, which is not considered a blister-rust infection area, the Viking was free of rust. There, the variety is being tested as regards its horticultural value for that region.

### In Canada

Through the courtesy of H. T. Güssow, Central Experimental Farm, Ottawa, Canada, 6 Viking plants were set out near the currant plantation of the Horticultural Division, containing a large number of red, white, and black currants, together with gooseberries. The Viking was planted within 8 feet of 2 *Ribes nigrum* plants and not far from a block of about 150 bushes of the same species. In 1932 Güssow reported the Viking (written communication) entirely free of rust. The two black-currant bushes were so heavily infected that they were partially defoliated, and the same condition held for the 150 plants, 50 feet away. In 1933, Viking, likewise, did not become infected; and the year later, Güssow stated (written communication) that, while general infection of currants in 1934 had not been severe in the locality, the Viking, although amply exposed to infection, continued to exhibit immunity. This testing with Canadian strains of white-pine blister rust, gave additional evidence that the Viking, whose resistance to Norwegian, British, eastern and western North American rust strains had been demonstrated, was undeniably a highly resistant variety.[10]

## DISCUSSION

In a recent publication (3, p. 15), in which the immunity of the Viking currant from white-pine blister rust under artificial conditions was reported, it has been pointed out that the utilization of the introduced rust-resistant Viking holds considerable promise, horticulturally, in areas outside of those regions where the growing of white pine is an economic factor. Because of the blister rust, the culture of currants and gooseberries has been restricted or prohibited in these control areas where the northern and western white pines, sugar pine, *Pinus lambertiana* Dougl., and other five-needle (white) pines are important. In those regions, however, where currants and goose-

[10] During the preparation of this paper, field data on the Viking for 1935 have been submitted to the author. There has been no record of any fertile infection on the Viking test plants for that year.

berries are permitted to be grown commercially, or cultivated in private gardens, the introduced Norwegian variety may be an acceptable addition to our horticultural catalog. Moreover, there should be a particular interest in the blister-rust-resistant Viking because of its breeding possibilities.

Currants are natives of cool, moist northern climates. In the United States, as stated by Darrow and Detwiler (1), they succeed best in the northern half of the country and east of the one hundredth meridian. In Norway, where the Viking (see description, 3, p. 3) has been cultivated many years, it still is regarded as the best commercial currant grown, and preferred by the Norwegians to other more recently developed and popularized varieties because it produces an abundant crop of fruit of good quality under their soil and climatic conditions. According to P. Stedje, horticulturist, Norway State Fruit Experiment Station, Hermansverk, Sogn, Norway, the average Viking crop for 1928–1929 (written communication) was 6 kgs. per bush or approximately 6000 kgs. per acre, which, for Norway, is considered a fine yield. Moreover, in Scandinavia, the foliage of the Viking is resistant to most fungus diseases. If, during the horticultural testing of the introduced variety in this country, it is demonstrated that Viking is resistant to the currant borer, *Sesia tipuliformis* Linn., and the imported currant worm, *Pteronidea ribesii* Scopoli., the plant will have added value for those currant regions where these insects are harmful. Fruit worms, particularly the currant maggot, *Epochra canadensis* Loew., (1, p. 2), are very serious pests of currants and gooseberries and have made the production of the fruit difficult in the Rocky Mountain and Pacific Coast States. The currant maggot, for which there is no known means of control, also occurs in some localities in the East, and can be rated along with moisture deficiency and summer heat, as one of the limiting factors in the successful cultivation of currants.

The Viking is now being distributed to horticultural stations in different parts of the country for the purpose of fully testing its horticultural value. Its resistance to heat and drought, as evidenced by the field tests reported in this paper, should make it an acceptable variety for planting in the regions of restricted rainfall, where the number of small fruits that can be grown are exceedingly limited. In the northern Great Plains area and in most parts of the upper Mississippi Valley, where the variety is to be tested, it cannot be expected to produce, however, the abundance or perhaps quality of fruit produced in northern humid climates. In Maine, for instance, where the Viking was tested for resistance to blister rust, the variety made excellent growth, which, with pruning, produced a heavy yield of fruit of good quality. Resistance to disease and insects should give the Viking added advantage for cultivation in dry regions.

The growing[11] of the immune Viking currant in white-pine-blister-rust control areas, without present or future danger to protected trees, is, however, open to question, because the seeds of the Viking are germinable and some of the seedlings may not be immune. The planting of large numbers of Viking currants in control areas might, therefore, establish numerous sources of germinable seed from which slightly susceptible plants might become established within these areas in increasing numbers. In view of the vigorous efforts of cooperating Federal and State agencies to control blister rust, by the suppression of all cultivated and wild currants and gooseberries, this might increase the difficulties and cost of maintaining control conditions within these pine-growing areas. It is an open question if the benefits to be obtained from introducing this plant into control areas would offset the increased difficulties and cost of control.

As has been pointed out (2), the origin of the Viking is unknown. The variety, which belongs to the group derived in part from the species *Ribes petraeum* Wulf., may be a hybrid and one of the many forms of the parent stock of the Red Dutch currant (6), cultivated in Europe since the end of the seventeenth century. If it originated by crossing a susceptible with a resistant variety, all the seedlings will not show resistance to rust. The manner of segregation of susceptible and resistant seedlings depends, of course, upon whether or not resistance is a dominant character in the parent. Preliminary inoculation results with open-pollinated Viking seedlings presented below indicate that resistance is dominant. On the other hand, Viking may have originated as a mutation within a variety. In the latter case Viking can be either homozygous or heterozygous for resistance, assuming resistance be dominant. If homozygous, all the seedlings will be resistant; if heterozygous, the seedlings from open-pollinated seeds will probably be segregating, part of them will be resistant and others susceptible.

An investigation is in progress to test the seedlings of the Viking to determine whether they likewise are immune from blister rust, or whether some susceptible seedlings will be produced. This investigation includes the testing of seedlings derived from open-pollinated seed where cross-pollination with nearby escaped susceptible red currants was not precluded, and seedlings derived from selfed-Viking seed. The Viking stock in the New England and New York test plots, have been used as sources for open-pollinated seed. In several instances cross pollination with nearby cultivated red currants in gardens, or escaped from cultivation, was possible.

Preliminary testing has been performed with seedlings derived from Viking seed collected in 1932 by P. Stedje, in the currant collection of the

[11] This statement on the growing of an immune currant in blister-rust-control areas was prepared by J. F. Martin, Senior Pathologist, Plant Disease Control, Bureau of Entomology and Plant Quarantine.

Norway State Fruit Experiment Station at Hermansverk. Cross-pollination was not debarred, so that it is likely that foreign pollen from susceptible varieties may have become involved. Among 314 Viking seedlings tested (1933 to 1935), 96 per cent of the open-pollinated seedlings when tested once were immune. Tests were repeated on 184 of these apparently immune seedlings and these were found to be immune on both trials. Additional testing has been performed with 65 of these immune seedlings; 43 were found to be immune on 3 trials, 19 immune on 4 trials, and 3 immune on 5 trials. Additional testing has been performed (1935) with 163 seedlings propagated from open-pollinated Viking seed derived from 12 different sources in New England. This lot of American seedlings showed 97 per cent immune plants. These preliminary experiments demonstrate the fact that we are correct in our assumption that resistance to blister rust is a dominant character in Viking. It will be interesting, indeed, to discover what percentage of resistant plants will appear among selfed-seedlings.

Coming back to the consideration of growing the Viking currant in the white-pine-blister-rust control areas, it is apparent that the problem is one dealing with seedlings of the highly resistant parent. As long as the immune variety is propagated exclusively by cuttings, the resistant characteristic will be perpetuated, but the seedling progeny is another matter. Had we been fortunate in discovering an immune variety, the seed of which would not germinate, there would be very little difficulty in utilizing Viking in the control problem. Tubeuf (7, p. 460) has reported the seed of the plants of the immune Rote Holländische, *Ribes petraeum* hybrid (*R. petraeum* × *rubrum*), with which he worked, as not germinable. Investigation of Tubeuf's plant might disclose the variety to be horticulturally desirable for cultivation in this country as well as valuable for breeding purposes.

SUMMARY

The resistance (near immunity) of Viking currant (Syn. Rød Hollandsk Druerips), an introduced horticultural variety from Norway, to eastern and western North American strains of white pine blister rust (*Cronartium ribicola*) was demonstrated for 973 plants in a comprehensive field test. This test covered a period of 3 years (1932 to 1934) and consisted of 28 plots distributed throughout blister rust areas in eastern North America—New England, New York and Ontario, Canada—and in Oregon in the Pacific Northwest. In no case were uredia or telia observed. Only necrotic flecks were observed on very young leaves; flecks did not appear on leaves fully mature.

Natural infection was augmented by artificial inoculations in the field, both in the East and in the West. The conditions for natural and artificial inoculation are described.

Results showing a high resistance of Viking to North American and British strains of blister rust corroborate the opinion of Norwegian pathologists and horticulturists who have not observed the Viking to become infected with rust over a period of many years' cultivation in Norway, where blister rust has been severe.

The Viking grew vigorously in northern New England where it made rapid growth on favorable soil sites. Extremely dry conditions were experienced on some of the test plots, but in spite of retardation of growth, the Viking survived, thereby showing its resistance to drought.

Preliminary tests with open-pollinated seedlings of the Viking propagated from Norwegian and American seed showed a very high percentage of immunity from blister rust. These results indicate that rust resistance is a dominant character in the parent.

A discussion of the growing of Viking in blister-rust-control and non-control areas is given.

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# THE SUSCEPTIBILITY OF CERTAIN WILD GRASSES TO TILLETIA TRITICI AND TILLETIA LEVIS[1]

GEORGE W. FISCHER[2]

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## INTRODUCTION

The incidence of *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn on hosts other than *Triticum* spp. has been reported from time to time in previous years. In 1922, Gaines and Stevenson (9) found $F_2$ plants of certain rye-wheat and wheat-rye hybrids bearing smutted heads; these proved to be due to *T. tritici*. In 1923 the same authors (10) found *T. tritici* occurring naturally, not only in wheat-rye hybrids, but also in rye, *Secale cereale* L., itself. To determine to what extent rye can serve as a host to *T. tritici*, Gaines and Stevenson (10) artificially inoculated the seed of 9 varieties of winter rye and 14 of spring rye, taking their inoculum from several wheat varieties. The results showed that 4 of the winter ryes and only 1 of the spring ryes were susceptible. The percentage of bunted plants was not high, varying from approximately 2 to 8 per cent. However, similar inoculations of $F_2$ wheat-rye seed (Turkey × Rosen) yielded approximately 75 per cent bunted plants. Further inoculation experiments indicated that *T. tritici* is weakly parasitic on ordinarily susceptible wheat varieties after passing one generation on rye. It is interesting to note that no infection of rye was secured with inoculum taken from artificially bunted rye. In conclusion these authors (10) suggest that possibly *T. secalis* (Cda.) Kühn and *T. tritici* "are in reality the same fungus growing on different hosts." In 1927, Ducomet (7) also reported the successful inoculation of rye with *T. tritici*, and suggested that *T. secalis* may be only a form of *T. tritici*. Three years later, Lobik (13) recorded the incidence of *T. levis* on rye, but did not consider the spores to be strictly typical of *T. levis*. Bressman, in 1931, (1, 2) succeeded in infecting 2 varieties of rye with "both species of bunt," obtaining 2.2 per cent infection. Of the several physiologic forms employed, Bressman's (4) form IX (*T. tritici*) was decidedly the most virulent.

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Bressman (2) also was of the opinion that *T. secalis* is merely a form of *T. tritici*. In 1932, Dillon Weston (5) further proved the susceptibility of rye to *T. tritici*, and in 1933 Nieves (14) confirmed the reports of the susceptibility of rye to both *T. tritici* and *T. levis*.

Our knowledge of the host range of *Tilletia tritici* and *T. levis* was further extended in 1931–32. The susceptibility of *Aegilops ventricosa* was demonstrated independently by Reichert (16) and by Gaudineau (11). At the same time Bressman (4) successfully infected *Lolium multiflorum* Lam. and *L. perenne* L. with *T. levis*. He used a mixture of 10 physiologic forms of *T. levis* and *T. tritici* for inoculum; however, only the former species appeared in the bunted heads.

Thus it has been shown that *Tilletia levis* and *T. tritici*, long supposed to be specialized to species of Triticum, can infect species of Secale, Aegilops and Lolium as well, although the latter hosts are much less susceptible. Up to the present time, no data have been published to further extend our knowledge of the host range of these two important wheat pathogens.

## PRESENT INVESTIGATIONS

On July 17, 1934, several bunted heads of crested wheatgrass, *Agropyron cristatum* (L.) Beauv., were received from Mr. H. M. Wanser, formerly Superintendent of the Adams Branch Experiment Station, Lind, Washington. Virtually every floret of each head contained a smut ball, which had the fetid odor often associated with bunt of wheat. Examination of the spores showed them to be morphologically similar to *Tilletia tritici*, and the collection was provisionally assigned to that species.

This incidence of what was apparently wheat bunt on crested wheatgrass incited a number of pertinent questions:

1. Is crested wheatgrass really susceptible to *T. tritici*, as the collection from Lind would indicate?

2. Will a long-lived perennial grass, such as crested wheatgrass, once infected, retain the mycelium of *T. tritici*, producing a crop of bunted heads each year? Bunt, or stinking smut of wheat has heretofore been reported only on cereals and grasses that are either annuals or short-lived perennials.

3. Can the bunt on crested wheatgrass serve as a source of infection of wheat, and if so, to what extent?

4. What other wild or cultivated grasses might also be susceptible to *T. tritici*?

5. Will *T. levis* also find congenial hosts in various of our wild and cultivated grasses?

In order to throw some light on these questions, rather extensive inoculation experiments were begun in the fall of 1934.

### WHEAT INOCULATIONS

To test the susceptibility of wheat to the bunt on created wheatgrass, and at the same time attempt to determine the form identity of the smut collection, the following standard differential varieties were inoculated in the regular manner: Hybrid 128 (C. I. 4512), Turkey (C. I. 6175), Ridit (C. I. 6703), Oro (C. I. 8220), Albit (C. I. 8275), Martin (C. I. 4463), Hohenheimer (C. I. 11458), White Odessa (C. I. 4655), and Hussar (C. I. 4843). The seed was planted in the uniform smut nursery at Pullman.

At the same time, seed of Hard Federation, a spring wheat known to be highly susceptible to most physiologic forms of *Tilletia tritici* and *T. levis* in the Pacific Northwest, was inoculated in flats of soil maintained at 11°–15° C. and the seedlings later transplanted to benches in the greenhouse, where they were grown to maturity.

### GRASS INOCULATIONS

The following species of wild and cultivated grasses were tested for susceptibility to *Tilletia levis* and *T. tritici*: *Agropyron cristatum* (L.) Beauv., *A. pauciflorum* (Schwein.) Hitchc. (*A. tenerum* Vasey), *A. repens* (L.) Beauv., *A. spicatum* (Pursh.) Scribn. and Smith, *A. subsecundum* (Link) Hitchc. (*A. caninum* L. Beauv.),[3] *Aegilops cylindrica* Host.; *Elymus canadensis* L., *E. condensatus* Presl., *E. glaucus* Buckl.; *Hordeum jubatum* L., *H. murinum* L., *H. nodosum* L.; *Secale montanum* Guss. Most of these are represented by more than one collection. This is especially true of *Agropyron cristatum* and *A. pauciflorum* of which there were twenty-two, and twenty-five collections, respectively. A total of sixty-four wild grass selections were used.

It has long been known that a marked increase in the precentage of infection with other cereal smuts may be obtained by inoculating seed from which the hulls have been removed (commonly spoken of as "dehulled" seed). In the present investigations both normal and dehulled seed of each of the sixty-four selections of wild grasses were inoculated.

The inoculum was of two sorts: (1) *T. tritici*, taken from the original collection of bunted crested wheatgrass from Lind, and (2) a mixture of *T. tritici* and *T. levis*.[4]

[3] According to Hitchock (12) our common bearded wheatgrass has erroneously been referred to *Agropyron caninum*, a distinct European species. His changes in nomenclature are being followed here.

[4] This mixture was supplied through the courtesy of C. S. Holton and consisted of three distinct forms of *T. tritici*, one of *T. levis* and a virulent hybrid between two smut species, which, according to Dr. Holton, has the pathogenicity of the *tritici* parent, and the morphology of the *levis* parent. These forms were selected because they are very distinct, pathogenically, and therefore, easily separated from a mixture.

Each grass selection was inoculated, therefore, in four ways: (1) Normal seed, with *T. tritici* from crested wheatgrass; (2) dehulled seed, with the same inoculum; (3) normal seed, with a mixture of *T. tritici* and *T. levis*; and (4) dehulled seed, with the same mixture. The inoculations were made by dusting the seeds rather heavily with the inoculum, after which they were planted in boxes and pots of sterilized soil maintained at a temperature of 10–14° C. When the hypocotyls were well above the soil, the containers were removed to the greenhouse. Plants thus inoculated during the winter were later transplanted to pots in the greenhouse. Plants inoculated in the spring were transplanted directly to the field. Since most of the grasses grown in the greenhouse during the winter months failed to head, they likewise were transplanted to the field.

## RESULTS

The data obtained from the wheat plantings show that the collection of *Tilletia tritici* from crested wheatgrass is capable of attacking certain wheat varieties, but is not especially virulent. Thus, of the differentials inoculated, only Hybrid 128 was badly smutted. The percentage of infection on this variety was 63.1, on Turkey, 8.53 per cent; the other varieties varied from a trace to 2 per cent. Sixty-five per cent infection was obtained on Hard Federation in the greenhouse. On the basis of the reaction of this collection of *T. tritici* to Albit, Hussar and Ridit, it could be assigned to Bressman's (3) physiologic form VIII, these varieties being essentially immune. The collection likewise conforms to Gaines' (8) physiologic form Tl, on the basis of the reaction to Hard Federation, and to the 9 winter wheat differentials.

The data obtained from the grass plantings described above are not complete, inasmuch as at least 30 per cent of the plants failed to head. This is especially true of the crested wheatgrass selections. Such plants remained in a bushy vegetative condition the entire summer. Culms were sometimes produced, but bore no spikes. However, at least a few plants of nearly every selection headed out so that some information was obtained concerning resistance or susceptibility to bunt. Thus, 15 of the 22 selections of crested wheatgrass proved to be to some degree susceptible to stinking smut of wheat. Twelve of these susceptible selections were susceptible to the collection of *Tilletia tritici* originally taken from crested wheatgrass. Seven selections were susceptible to 1 or more of the 5 forms comprising the mixture of *T. tritici* and *T. levis* described above. Microscopic examination of the bunted heads showed none of the 7 selections to be susceptible to *T. tritici*, whereas 6 were susceptible to *T. levis* or the hybrid form with the *levis* morphology. The form identity in all cases can be determined

only by inoculating back to the 9 differential wheat varieties. Since so many of the plants of crested wheatgrass failed to head, reliable percentages of infection cannot be calculated. For the present it is sufficient to point out the susceptible nature of crested wheatgrass. Percentages of infection, varietal resistance and susceptibility, and physiologic form identity will be determined by investigations now in progress.

The data from the plantings of slender wheatgrass, *Agropyron pauciflorum*, are more reliable, since the majority of these plants headed out with very little of the suppression of flowering observed in crested wheatgrass. Sixteen of the 25 selections of slender wheatgrass proved to be more or less susceptible to bunt. Of these, 11 were susceptible to the original collection of *Tilletia tritici* from crested wheatgrass; 8 were susceptible to 1 or more of the 5 forms of *T. tritici* and *T. levis* in the mixture used for inoculum. Microscopic examination of the spores showed that bunted heads of 6 of the 9 varieties were due to *T. tritici*, and 5 to either *T. levis* or the hybrid form. Three selections were susceptible to both smut species in the mixture. There was an indication of rather wide varietal differences in susceptibility among the selections tested, since 8 selections were bunt-free, while 17 showed 5 to 50 per cent infection. A few selections are apparently quite susceptible, while several are highly resistant, or immune, at least to the physiologic forms of *T. tritici* and *T. levis* used in the present investigation.

In addition to crested wheatgrass and slender wheatgrass, 2 collections of bearded wheatgrass, *Agropyron subsecundum*, were tested and found to be susceptible to *Tilletia levis* and *T. tritici* when artificially inoculated with the mixture of the two smut species.

Of the three species of Hordeum tested, only *H. nodosum* proved to be susceptible to bunt. Here again the degree of susceptibility is a matter for conjecture, since only 2 plants survived competition in the greenhouse and matured when transplanted to the field. One of these bore only smutted heads, having been inoculated with the crested wheatgrass collection of *Tilletia tritici*.

The morphology of the bunted heads is usually somewhat different from that of the normal heads. In the case of crested wheatgrass the bunted heads are darker green, shorter, more compact and more plump than the normal ones (Fig. 1, C and D). This effect is due, in part, at least, to the fact that nearly every floret contains a plump, dark green smut ball or "sorus," which spreads the palea and lemma apart in a way that a developing seed would not do. As the heads pass maturity the smut balls shrink and fade somewhat, but, while still green, the bunted heads are usually in marked contrast to the normal ones, thus making recognition

FIG. 1. A and B. *Agropyron pauciflorum*. A. Normal spike. B. Bunted spike. C and D. *A. cristatum*. C. Normal spike. D. Bunted spike.

FIG. 2. A and B. *Hordeum nodosum*. A. Bunted spike (due to *Tilletia tritici*). B. Normal spike. C and D. Bearded wheatgrass, *Agropyron subsecundum*. C. Normal spike. D. Bunted spike.

as easy as in the case of bunt of wheat. This is somewhat less true when the bunted heads are due to *Tilletia levis* than to *T. tritici,* since the former generally produces more elongate smut balls, which do not spread the palea and lemma so much. This and other morphological differences between *T. levis* and *T. tritici* have long been observed in bunt of wheat (15).

The recognition of bunted heads of the other susceptible grasses is essentially the same. In the case of slender wheatgrass the bunted heads, in comparison with the normal (Fig. 1, A and B), are more dense and contracted. In some cases, especially when due to *T. levis,* the smut balls are quite elongate, often projecting several millimeters beyond the tips of the palea and lemma. The area exposed to the sun becomes reddish purple, with the result that these smut balls superficially resemble so many ergots. Such bunted heads can easily be recognized from a distance of several feet.

The presence of the long awns on *Agropyron subsecundum* somewhat modifies the appearance of the bunted heads, but such are almost as conspicuous as described for slender wheatgrass. The bunted heads are similarly more dense than the normal ones (Fig. 2, C and D).

On *Hordeum nodosum* the bunted heads are scarcely more conspicuous than normal ones. There is only one smut ball per spikelet, since, even in normal heads, the lateral florets of each spikelet are abortive. Sometimes only about half of the spikelets bear smut balls (Fig. 2, A and B), but in such cases no seeds have been found in the bunt-free spikelets.

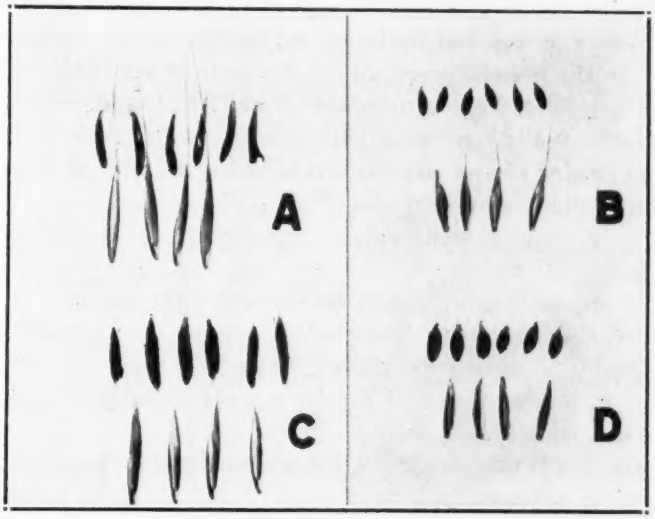


FIG. 3. Smut balls (upper) and normal seeds (lower). A. Bearded wheatgrass. B. Barley grass, *Hordeum nodosum.* C. Slender wheatgrass. D. Crested wheatgrass. Natural size. The slender wheatgrass smut balls contained only *Tilletia levis;* the others contained only *T. tritici.*

Feucht (7) claims that the shape of the smut ball or sorus is influenced more by the normal shape of the seed of any particular wheat variety than by any inherent tendency of *Tilletia levis* to produce elongate sori, or of *T. tritici* to produce short, thick, or rounded sori. Whether this holds true for wheat is perhaps debatable, but it is seen that in the case of the grasses described above, the shape of the smut ball was decidedly influenced by the grass in question. Smut balls on *Agropyron cristatum* tend to be relatively short and thick and rounded, although more so for *T. tritici* than for *T. levis*. Smut balls on *A. pauciflorum* and *A. subsecundum* tend to be decidedly elongate, although sometimes more so for *T. levis*. Figure 3, A – D, illustrates the relative size and shape of smut balls and normal seeds of *A. subsecundum, Hordeum nodosum, A. pauciflorum* and *A. cristatum,* respectively.

## DISCUSSION

It cannot be safely stated yet just how much significance may be attached to the susceptibility of these wild grasses to *Tilletia tritici* and *T. levis*. The criticism may be advanced that the susceptibility demonstrated for certain of these wild grasses was brought about by conditions artificially optimum, and, therefore, such infection may not be a reliable indication of what might be expected under natural conditions. However, the original collection of bunt on crested wheatgrass from Lind, Washington, did not result from artificially inoculated seed, nor subsequently cared for under recognized optimum conditions of soil temperature and moisture. In the present investigation the marked predominance of heads in which every floret bore a well-formed smut ball, and the fact that an infected plant usually bore only bunted heads leads the writer to believe that certain strains of crested wheatgrass and of slender wheatgrass might be quite susceptible under field conditions. Field plantings are in progress in which only normal seed will be inoculated and tested under field conditions.

It would appear, from the data taken during the summer, that hulling the seed does not necessarily promote infection of some grasses. Thus, of 30 bunted plants of crested wheatgrass, 18 resulted from inoculated normal seed, but of 48 bunted plants of slender wheatgrass only 13 resulted from inoculated normal seed.

It remains for future investigations to demonstrate the extent to which the presence of perennially bunted grass plants could be a factor in the control of wheat bunt, as well as a limitation factor in the culture of forage grasses. Field surveys should be made to determine the extent to which bunt occurs naturally on crested and slender wheatgrass.

SUMMARY

Specimens of crested wheatgrass, *Agropyron cristatum,* in which essentially every floret contained a smut ball, have been found in field plantings, and this smut has been shown by its morphology and by inoculations on wheat to be *Tilletia tritici.* Of the 9 winter wheat varieties tested, Hybrid 128 was the most susceptible, showing 63.1 per cent bunted heads. Hard Federation, the only spring wheat tested, yielded 65 per cent bunted heads.

Sixty-four collections of wild grass species of the genera Agropyron, Elymus, Hordeum, Aegilops, and Secale have been tested for susceptibility to *Tilletia levis* and *T. tritici.* These included 22 selections of crested wheatgrass, and 25 of slender wheatgrass, *Agropyron pauciflorum.*

Crested wheatgrass, slender wheatgrass, and bearded wheatgrass, *Agropyron subsecundum,* were found to be susceptible to certain physiologic forms of both *T. levis* and *T. tritici. Hordeum nodosum* was susceptible to *T. tritici.*

Of 22 selections of crested wheatgrass tested, 13 proved to be in some degree susceptible to *T. tritici,* 6 to *T. levis* and 3 to both species.

Fourteen of the 25 selections of slender wheatgrass tested proved to be in some degree susceptible to *T. tritici,* 5 to *T. levis,* and 5 to both species.

There is evidence of considerable varietal resistance and susceptibility among the selections of crested and slender wheatgrasses inoculated with *T. tritici* and *T. levis.*

Species of Secale, Aegilops, Lolium, Agropyron, and Hordeum have thus far been shown to be to some degree suceptible to bunt of wheat.

DEPARTMENT OF PLANT PATHOLOGY
STATE COLLEGE OF WASHINGTON
PULLMAN, WASHINGTON

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# THE STUNT[1] DISEASE OF JAPANESE RICE, THE FIRST PLANT VIROSIS SHOWN TO BE TRANSMITTED BY AN INSECT VECTOR

SABURO KATSURA

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## INTRODUCTION

The historic outbreak of the stunt disease of the rice plant in Japan occurred in the 30th year of *Meiji*, 1897, in the leading rice districts of the prefectures Shiga, Kyoto, Hyogo, Okayama, Hiroshima, and Shizuoka. Previously, minor outbreaks had been known locally, since the disease was first discovered in Shiga prefecture in 1883. This disease is transmitted by a leaf hopper known as *Tsumakuro Yakobai,* or *Nephotettix apicalis* Motsch. var. *cincticeps* Uhl., and was the first case noted of a biotically transmitted plant virus.

A very early record of severe ravages of leaf hoppers is found in the history of the Japanese famines, which tells of a tragic famine in the 18th year of Kyoho, 1733, when the fertile rice fields of 46 clans of feudal Deimyos were attacked. History records 12,000 people dying of starvation—nearly 5 per cent of the total Japanese population at that time.

When the serious outbreaks of the stunt disease occurred toward the end of the last century, Japan had already begun scientific studies of plant diseases and plant-pest control, but so severe and subtle was the attack of this disease that early science could do little to combat it. Although the people no longer ran the extreme risks of famine, the balance of the nation's food supply was seriously upset.

The menace to the nation's staple food impelled early plant pathologists to initiate strenuous campaigns against the disease that had so suddenly become a major problem.

## HISTORICAL REVIEW

The oldest published scientific record concerning the stunt disease appeared in one of the early government gazettes (10) while the disease was attracting little attention. This was written as a formal reply to an interrogation by the Government of the Kyoto prefecture. A paragraph from this report follows—this paragraph being reprinted in an article by Fukushi (8).

"Small insects locally known as 'Unka' (leaf hopper) attacked the field in *Kii-gun, Kyoto.* By their mass attack on the culms and leaves of the rice plant these insects interfere with the normal growth of the plant. Some

[1] Commonly called dwarf disease, but the writer prefers the name stunt disease.

people suspect that the insect causes the stunt disease in this fashion and point to the punctures usually found in the leaves of stunted rice. But it should be remembered that sap sucking insects in general would naturally attack the tender tissue of diseased plants. The insect may add to the damage of the stunt disease, but the cause of the disease should by no means be attributed to its attack."

The author of this comment has never been made known; however it is believed that the report was issued as a sort of warning to growers who, from their observation of the active presence of the leaf hoppers, were prone to conclude that the latter were the responsible factor.

The relation of leaf hoppers to the stunt disease of rice was first experimentally proved in very early days by a local grower, Hatsuzo Hashimoto of Shiga prefecture, the center of the disease-stricken area. His little-known work is no small contribution and actually deserves to be considered as one of the very important discoveries. It was because of his failure to publish the results of his studies connecting leaf hoppers with the disease that he is today scientifically unknown, yet his findings more than anything else directed research toward the true solution of the problem.

Hashimoto, according to Ishikawa (9) started his experiments as early as 1883, and the following year discovered the relation between leaf hoppers and the disease. In his investigations he planted rice seedlings in a glass container and put in some leaf hoppers, covering the container with a piece of Victoria lawn to keep the insects within.

The experiments were carried on for two years subsequent to his discovery, so that he could be absolutely sure that his insect was guilty. The experiments were somewhat rudimentary but the basic concept was unique and won a position of paramount importance to the study of this specific virus disease of the rice plant. The attention of other investigators was directed toward various edaphic factors, and at first little notice was taken of Hashimoto's studies.

The Government Gazette (10) printed another misleading paragraph, stressing the importance of soil treatment as a control measure. The production of iron sulphide as the result of oxygen depletion in the soil was postulated to be the chief cause of the checking of normal growth. In order to oxidize the iron sulphide formed in the soil, the report recommended the removal of stagnant water from poorly drained fields, insolation of the soil, and application of unslaked lime.

Much discussion regarding the cause of the disease was entered into by various people, each pointing out different factors, such as poorly-drained fields, drought, low temperature of irrigation water, excess fertilizer, etc.

At this time, so prolific of hypotheses, Hashimoto was quietly bringing his experiments toward a positive result, and finally discovered the impor-

tant clue to the cause of the disease. Perhaps, because of his modesty at not having had pathological training, he made no formal report to any institute and the species of leaf hopper with which he worked was never made known.

In 1883, the government of Shiga prefecture appointed K. Takata to take charge of several experimental plots located in various part of the prefecture. In their first study on transmission of the disease through the soil, Takata and his colleagues definitely ascertained that iron sulphide deposited in the soil bears no relation to the occurrence of the disease.

Takata's attention then was turned to the leaf hoppers, which circumstantial evidence indicated had some possibilities, and that Hashimoto's contemporaneous studies stressed. The first leaf hopper that came to his notice and that he believed to be responsible for the cause of the disease was "Monyokobai," which in later years assumed another name, "Inazuma Yokobai," or *Deltocephalus dorsalis* Motsch. A summary of Takata's work (20) was published in December, 1895 and 1896. This was the first paper in which the etiological significance with respect to the stunt disease was ascribed to an insect pest. In an incredibly short time the main conclusion of the report became known throughout the disease-stricken areas where the spraying of coal oil on the soil became the vogue in checking the newly reported pest.

Takata's papers, although not known to be erroneous in their conclusions, are of historical importance because of the widespread interest they aroused among pathologists. At first his claims found apparent confirmation, but later work by his junior colleagues definitely refuted them. The reported etiological relation between the leaf hopper, *Deltocephalus dorsalis,* and the stunt disease of rice was found to be incorrect.

It is apparent today that the guilty leaf hopper, *Nephotettix apicalis,* although unnoticed, undoubtedly was present in Takata's material, imprisoned in his test chamber; otherwise, no stunt disease could have been produced.

In 1895, the year in which Takata's first report was issued, a branch of the Imperial Agricultural Experiment Station was established in the Shiga prefecture and Takata was appointed its first director. The Shiga station in the first year collected every suspected species of leaf hopper, which belonged to 8 species, including Takata's *Deltocephalus dorsalis,* which was still the center of attention.

In 1898, the station removed 3 species of leaf hopper from the suspected list. The results of this work were published the following year as the first official report of the Shiga-Ken Agricultural Experiment Station (15). The report summarized the general plan of the study of insect pests, concluding:

"The stunt disease of rice is caused by the leaf hoppers, *Nephotettix apicalis* Motsch. var. *cincticeps* Uhl., *Deltocephalus dorsalis* Motsch., *Thamnotettix tobae* Mats., *Cicadula fascifrons* Stal., and *Zygina limbata* Mats., while none of three others, such as *Delphacodes striatellus* Fall., *Tattigonia viridis* L. and *Nisia atrovenosa* Leth. was found to be responsible for the occurrence of the disease."

Undoubtedly, the disease was believed at this time to be caused by these named leaf hoppers.

It was, thus, in the 1898 experiments that the really guilty leaf hopper, *Nephotettix apicalis,* was first detected and put on the black list together with 4 others.

In 1900, the station issued Report 2 of the insect pest series, which tells of the narrowing down to *Nephotettix apicalis* as the only leaf hopper capable of producing the true stunt disease of the rice plant. With the isolation of the responsible leaf hopper from the 8 species, the very complex problem seemed to have reached its solution.

The actual causal agent that brings on stunting of rice is as yet unknown. As early as 1900, however, the investigators of the station somewhat hastily concluded that the newly singled out leaf hopper was the etiological factor.

In 1901, N. Takami (19), then the principal plant pathologist in charge of the station, reported his experiments, which he carried on for 6 years, commencing in 1896. In this publication he described the leaf hopper as the sole causal factor, a claim that he later withdrew.

Meanwhile the station (15) continued to extend its insect-pest studies, which, in 1906, reached the sixth report in which the experiments of the previous year worked out under the guidance of Takami were discussed.

This report repeated the hypothesis that Takami outlined in his 1901 paper, and strongly attempted to define the etiology of the disease with his brief that the insect injury was the sole cause of stunt of rice. He based his contention upon his observation that the disease actually was produced by the leaf hopper in his tests carried on for years. This specific leaf hopper, he further stated, was injurious only when it was an inhabitant of a diseased area, while those individuals collected from other places were incapable of producing the disease. In this same year, and shortly after issuing the sixth report, he withdrew his original claims, which his subsequent work had shown to be completely in error.

Takami began to grow suspicious that his leaf hopper acted as a carrier of the disease. It was a bitter state of affairs for him, as his long-held hypothesis had to be altered almost over night. Nevertheless, with such elimination of errors, the studies found their way toward the true solution. Takami is unquestionably one of the men who have outlined important

chapters in the story of the stunt disease of rice. The insect-pest series Nos. 7 and 8, issued in 1908, (15) record many painstaking studies undertaken by the investigators of the Shiga-Ken Agricultural Experiment Station.

The fact about the subtle biological relation regarding the incidence of the disease was first uncovered when the same species of leaf hoppers collected at Tokyo and northern districts outside of the diseased area acquired infectious power when reared on the diseased plants for a period of 5 days, or more. It was in 1906 that the possibility of the leaf hopper acting as an intermediate host was sensed, and, immediately, relevant experiments were started. By 1908, the results yielded substantial confirmation. Meanwhile the Nishi-ga-Hara Imperial Central Agricultural Station situated near the city of Tokyo carried out experiments on the same subject.

As to the stunt disease of rice, the Nishi-ga-Hara station had produced no reliable data up to 1905, when the current hypothesis was that suggested by Takami of the Shiga station. His theory, attributing the cause of the disease to leaf hoppers, was received with much skepticism at Nishi-ga-Hara. The men of the station, under the leadership of H. Ando, decided upon a more thoroughly controlled plan of experimentation. In 1905, the Nishi-ga-Hara station received from Shiga prefecture some stunt-diseased rice plants on which disease-free leaf hoppers, native to Tokyo, were reared in order to produce infected offspring. The offspring, newly hatched, were found to be infectious upon feeding on healthy plants, but another group, whose disease-free parents had fed on healthy plants, showed no sign of the disease. The following year the Nishi-ga-Hara station confirmed the fact that the leaf hopper at Tokyo acquired the infective power if allowed to feed on diseased plants for about 15 days (14).

Another interesting experiment carried out at the Nishi-ga-Hara station concerned the exhaustion of the infective power. The infective leaf hoppers collected at Shiga prefecture were fed on healthy plants that were repeatedly replaced with fresh healthy ones at intervals of from 5 to 7 days. This prevented the leaf hoppers from sucking any diseased juice that might have resulted from infection by their own saliva. As a result of this test it was shown in 1906 that the first progeny was capable of producing the disease, but the second and third were unable to do so. This result, of greatest importance, was announced by the Nishi-ga-Hara station in its report in 1909 (14). The conclusion was then drawn that the stunt disease of the rice plant was of unknown and of mysterious origin, the causative agent of which evidently was carried by the leaf hopper, *Nephotettix apicalis* Motsch. var. *cincticeps* Uhl., which performed the rôle of an insect vector. Perhaps the Nishi-ga-Hara central station was the first to prove

the fact, although the Shiga branch station reported the confirmed data in 1908 (15).

Japan's first report telling of a biotically transmitted stunt disease of rice, now known to be a virosis, thus appeared in positive language before the confirmed report of the curly top of beets in the United States. The latter was the first case in literature other than Japanese of a plant virus shown to be transmitted by an insect vector, in this case *Euttettix tenellus* (Baker). The association of *E. tenellus* (Baker) with curly top of sugar beets was first reported by E. D. Ball (2) in 1905, but definite proof of the relation of the insect to the transmission of the disease was first shown by H. B. Shaw (16) in 1910, and later by R. E. Smith and P. A. Bonquet (17, 18) in 1915, and by others.

### RECOGNITION AS A VIRUS

The most active discussion then arose regarding the causal agent of stunt. Until comparatively recent years, its type was not understood, but G. Daikuhara in 1902 (3) reported that the insect injury is not the major cause of the disease, and in 1904 (4), he made an unusual prediction regarding the problem. He studied the biochemistry of stunted rice and regarding the nature of the disease stated that it was allied to the sereh disease of sugar cane, to the mosaic disease of tobacco, and very likely to the stigmonose of carnations. In 1905, T. Yonemaru and K. Aso (21) of the Kyoto Agricultural Experiment Station expressed an opinion similar to Daikuhara's, but at that time no reliable data as to the etiology of the disease was available. In 1910, when Andô (1) made his conservative claims in announcing *Nephotettix apicalis* as the carrier of the disease, he stated that the disease was a mysterious one and entirely new to Japanese pathology.

The Nishi-ga-Hara central station's annual reports for 1913–1915 issued in 1917 (14), claim the presence of certain protozoa in the diseased tissues of the plant as well as in the blood of the insect vector. This theory was refuted by M. Kasai (11) of Ohara Institute whose research work in 1924 resulted in finding the Nelson bodies associated with the disease, but no protozoa, he strongly stressed, were to be seen. The bodies, which he called Nelson's type, were later found to be apparently different from those described and figured by Nelson.

The stunt disease of rice became the foremost subject in Japan, and a number of agricultural experiment stations, scattered in various localities, commenced to carry on research work on it. It was assumed that the causal agent of this disease, too small to be microscopically visible, belonged to the virus disease group when considering the symptoms of the disease, occurrence of X bodies, and the mode of transmission.

In 1931, Fukushi (5) reported that certain intracellular bodies were invariably present in the chlorotic tissue of the diseased leaf. His hypothesis is at the present time the most widely accepted one in Japan. Recently his work, mainly carried out in the laboratory at Sapporo University, Hokkaido, Japan, was published (7), describing his improved technique in English for the benefit of western readers.

So far as is known, there has been reported in no other country incidence of the stunt of rice described for Japan. L. O. Kunkel, then at Boyce Thompson Institute, Yonkers, N. Y., was the first investigator in the United States who took an interest in the stunt disease of rice. It was he who in 1926 pointed out the fact that the stunt of Japanese rice apparently belongs to the viroses. He discussed the disease with Fukushi on the occasion of the latter's visit to the United States in 1926. It was shortly after the writer's arrival in Washington, D. C., that he was requested by Kunkel to make an English translation of Takami's paper on the stunt of rice, mentioned in an early paragraph. Kunkel (12) cited the translation in his report, "Aster Yellows," made in 1926, giving recognition to the fact that the stunt disease of Japanese rice was the first known case of a virus disease transmitted by an insect vector.

## THE VIRUS WITHIN THE VECTOR

Up to the present, studies have shown that there are approximately 40 species of insects actually capable of transmitting virus diseases from plant to plant. In some cases the insect vectors do not carry the disease just mechanically from plant to plant, but the virus sucked by an insect from a diseased plant seems to be increased by some form of multiplication in the bodies of the carriers. These vectors are mostly cicadellids and aphids; some of them are not yet specifically identified. A comparatively small number of thrips and whitefly vectors also have been reported. Determination of the mode of virus transmission is difficult, such determinations being likely to be largely influenced by individual judgment, since the course of the transmission involves many a complicated bridge. Therefore, we may naturally expect to find some reasonable changes and additions to the category of insect vectors in future investigations, as more data are acquired.

Kunkel (13) expressed his opinion regarding the difficult task of drawing a distinct line between the mechanical and biotic methods of virus transmission from the data that had been obtained. He stated that "Viruses that are not mechanically transmitted, or at least not easily so transmitted, are spread by biological carriers only." According to this, the stunting of rice in Japan is a good example of a biotically transmitted virosis. In 1904, Daikuhara (4) reported the failure to transmit stunt by hypodermic

injections of the diseased sap. Various mechanical means of transmission, including infection with the ground-up bodies of infective leaf hoppers, attempted in 1911 at the Nishi-ga-Hara station, also gave negative results. Fukushi's (7) recent attempts with mechanical transmission in many ways also have failed. As a result of his extended study of stunted rice, he is now impressed strongly with the fact that the attack of this specific virus is possible only when transmitted through the intermediacy of the specific biological carrier.

He reports having carefully sought a clue in various suspected organs in the body of the viruliferous insects. He searched for inclusion bodies, which, in his opinion, might occur within the body of the vector, since he detected the so-called chlamydozos-like bodies in the intercellular bodies found in the tissue of the diseased plants. But, after repeated studies, he failed to find any inclusion bodies, any microorganisms of etiological significance, or any visual evidence of the presence of the virus in the salivary gland and alimentary canal, the egg follicles, the ovarian tubules, the mycetome, or other organs. Nevertheless it is logically conceivable that the virus in some form may be detected in the insect vector. This problem still awaits more extended research.

While it is not known just how the virus is carried in the body of the insect vector, some facts hitherto unknown have been determined by recent studies on the disease carrier. Fukushi's (6, 7) discovery that the virus is carried through the eggs is no doubt of outstanding importance. Such insects are born to the destiny of infecting plants with the virus they carry. He reports that it is not in the egg stage but prior to release of the egg that the progeny receives the virus. The eggs are not infected after they are deposited on the leaf blades. An astonishing phenomenon must have taken place in the body of the viruliferous insect; the attack of the virus in all likelihood occurs during the very early stage of egg formation in the ovary. The assumption that the virus enters the eggs has been sufficiently supported by Fukushi's researches. He believes they are infected in the ovaries and states that "Ovaries, therefore, must not be left out of consideration." This remark may bear much significance and be strikingly near the truth.

Real advancement in scientific research on these delicate biotic carriers is now destined to be made along entirely new lines. The ultramicroscopic entities, either living or non-living, that are now called viruses, present fascinating and important problems that challenge the intelligence and shrewdness of workers in the fields of phytopathology and entomology.

BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

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# COMPARISON OF DERIVATIVES FROM DISTINCTIVE STRAINS OF TOBACCO-MOSAIC VIRUS[1]

FRANCIS O. HOLMES

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It recently has become evident that there exist closely related strains of many viruses. The strains of any one virus possess many properties in common, generally having, for example, essentially the same host ranges, similar thermal inactivation temperatures, and common antigenic properties. They usually differ from one another only slightly, as in symptom expression on host plants. Strains of one virus differ as a group from strains of another virus in respect to most of their characteristics. An essential feature of virus strains is that they may be transmitted serially without loss of distinctive characteristics. It is generally assumed, and in some cases known, that they have been derived from preexisting parent strains (3, 4, 5, 6). The extent to which strains transmit their distinctive characteristics to variants arising from them is not known. Experiments were undertaken for the purpose of determining this for tobacco-mosaic virus (*tobacco virus 1*) in the case of 2 previously described strains, the *masked*, and the *distorting* from which the *masked* originated.[2] The experiments were designed to determine whether or not yellow-type strains derived from the *masked* strain of tobacco-mosaic virus would be closely comparable with those derived from the *distorting*, without this previous transformation into the *masked* strain.

## STRAINS OF TOBACCO-MOSAIC VIRUS USED AS SOURCES OF DERIVATIVES

*Masked* tobacco-mosaic virus differs from *distorting*, an ordinary field-type strain from which it was derived, by such characteristics as failure to induce mottling and distortion in infected plants of *Nicotiana tabacum* L. and a number of other species that are typically mottled and distorted by field-type virus. It also differs from *distorting* virus by a tendency to invade young leaves less extensively in *N. tabacum*, as well as to remain

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[2] For the sake of conciseness, the italicized words "*distorting*" and "*masked*" will be used as names of the distorting and masked strains, respectively. This will aid in brevity and help to avoid the false implication that masked virus is characterized by inability to produce symptoms in all hosts mentioned. The name was chosen originally because this strain produces only masked symptoms in *Nicotiana tabacum;* in certain other species, as in *N. glutinosa*, it produces conspicuous symptoms.

localized in the inoculated leaf in young plants of *N. rustica* L. It possesses the basic characteristics of tobacco-mosaic virus; high thermal inactivation point, resistance to inactivation when stored dry or in frozen juice, and ability to infect at high dilution (3, pp. 855–858). Furthermore, Chester (1, 2) has shown that it reacts with the sera of animals previously injected with distorting-type tobacco-mosaic virus, giving a characteristic neutralization reaction (1, p. 1200) as well as complement fixation and precipitin reactions (2, p. 694). Occasional yellow spots appearing on otherwise symptomless leaves infected with *masked* virus are potential sources of yellow-type strains (3, pp. 845–846). Sometimes these yellow spots, like those appearing on plants affected by ordinary tobacco mosaic, become semi-necrotic or fully necrotic in type. They will be referred to here merely as yellow spots, since they are usually yellow when first seen. Since the time of its derivation from *distorting* virus, the *masked* strain has been transferred frequently and preserved at times in frozen juice samples or dried leaves. As an indication of the probable degree of purity of the stock in hand at the beginning of the present experiments, an outline will be given of recent transfers. Virus was transferred to plants of *N. glutinosa* L. and isolated again from a single necrotic primary lesion. It was then transferred to *N. tabacum* and carried through 12 serial passages in that species, its presence in these symptomless plants being confirmed from time to time by inoculation of *N. glutinosa* plants. Subsequently it was transferred to plants of *N. langsdorffii* Weinm., and carried through 5 serial passages in single necrotic lesions. Then it was transferred again to plants of *N. tabacum* and preserved in dried leaves. Since even one transfer from a necrotic lesion in *N. langsdorffii* or *N. glutinosa* suffices to separate intentionally mixed strains, it is believed that the final stock of *masked*-strain virus represented essentially a pure line. It is understood that very small amounts of new derivative strains may have appeared in any transfer, and may be represented in the final sample, but there has been no indication of their presence in this one, and no reason to believe that they need be taken into account.

The *distorting* strain (3, p. 847) has the properties of typical tobacco-mosaic virus; these are too well known to be recounted here. The name *distorting* was applied to this particular isolated strain to distinguish it from other isolated strains, also of distorting type, and possessing the generally recognized properties of typical tobacco-mosaic virus, but differing from the strain in question by producing more stunting in *Nicotiana tabacum*, flecks of necrosis in invaded leaves, less distortion of leaves, or other distinctive symptoms. The stock of *distorting*-strain virus used in these experiments was one that had been obtained after repeated isolation

from single lesions in plants of *N. glutinosa* infected with highly diluted samples of juice of mosaic plants.

### DERIVATION OF YELLOW-MOSAIC STRAINS

New yellow-type strains were derived from the *masked* and *distorting* strains as follows. Sets of young plants of *Nicotiana tabacum* var. Turkish were inoculated with the previously purified stocks of tobacco-mosaic virus of the *masked* strain and the *distorting* strain respectively, from tobacco plants that had not been diseased long enough to show yellow spots. Healthy control plants were retained in numbers equal to those in infected

TABLE 1.—*Summary of experiments on derivation of yellow-type strains from two known strains of tobacco-mosaic virus*

| Experiment number | Strains of tobacco-mosaic virus, used as sources of yellow type derivatives | Yellow-type strains isolated | | | Failures to obtain yellow-type strains | Totals of attempts to isolate new strains[b] |
|---|---|---|---|---|---|---|
| | | Systemic, fully invasive | Systemic, not fully invasive | Localized only[a] | | |
| 1 | Distorting strain | 23 | 5 | 3 | 13 | 44 |
| 2 | Masked strain | 0 | 4 | 4 | 17 | 25 |
| 3 | " " | 0 | 7 | 3 | 31 | 41 |
| 4 | " " | 0 | 2 | 2 | 36 | 40 |

[a] In addition to those recorded in this column, 7 nonsystemic yellow-type strains were obtained in exp. 1, 4 in exp. 3, and 1 in exp. 4, in sets of transfers that also yielded systemic yellow-mosaic strains.

[b] For each attempt to derive a new strain of virus, 24 Turkish tobacco plants were inoculated, by two pin punctures in each, from a yellow or necrotic spot on a plant infected with the indicated one of the two strains of tobacco-mosaic virus used as sources.

sets; no mottling symptoms, and no yellow spots appeared on these uninfected plants. As soon as yellow spots appeared on the inoculated plants, isolation of new strains was attempted. Sometimes more than one yellow spot appeared on a single infected plant, but only one was used in each case. From each tested yellow spot, transfers were made by a method essentially like that of Jensen (4, p. 965) using a sterilized No. 00 insect pin. One leaf of each of 24 healthy young tobacco plants received two inoculation punctures near its base.

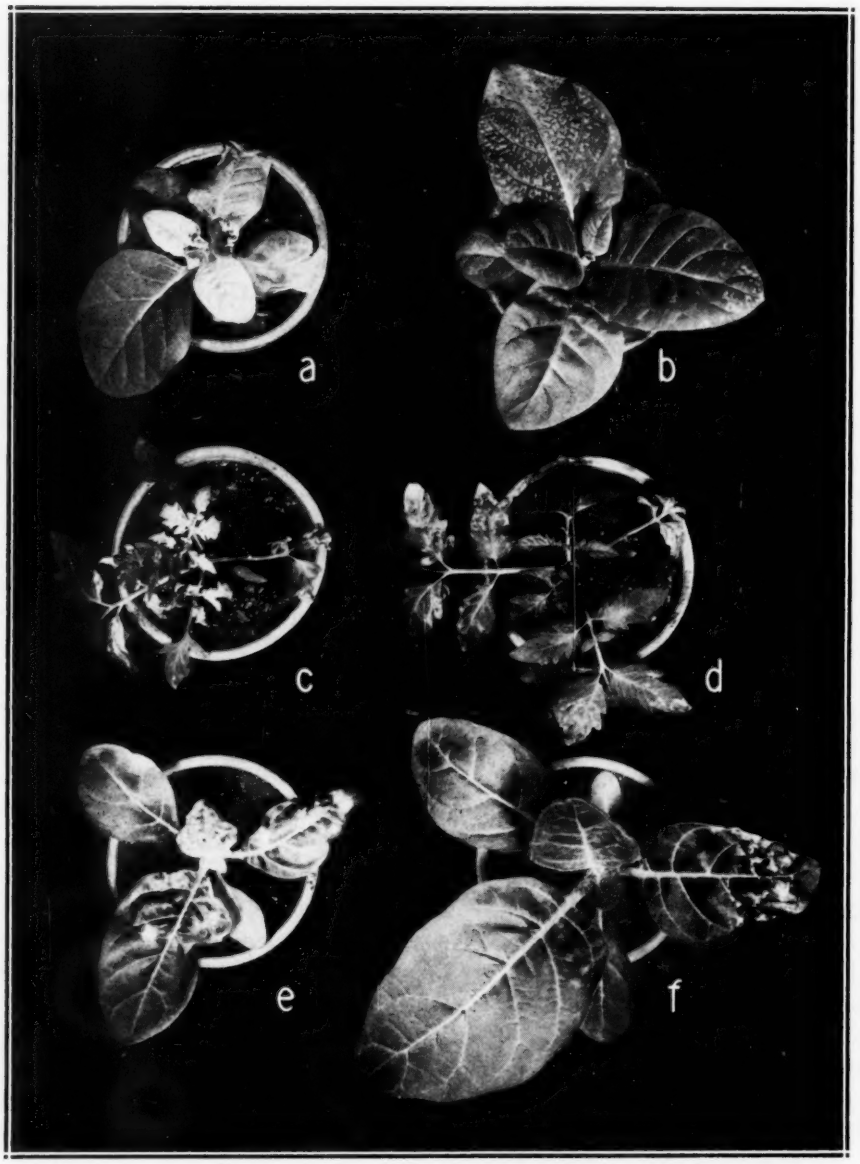
In table 1 are shown the summarized results of several experiments. Yellow-type strains were isolated from 31 separate tobacco plants previously infected with *distorting*-strain virus. These yellow-mosaic strains showed considerable variation in invasiveness, that is, in ability to invade

and affect the younger leaves of infected plants, as well as in degree of yellowness of affected leaves, tendency to produce necrosis, and other characteristics. The variation was similar to that recognized previously from reports of Jensen (4, 5) and McKinney (6) for yellow-mosaic strains derived from field-type tobacco-mosaic virus. Of the 31 yellow-type strains, 23 showed a sufficient degree of invasiveness to cause puckering of young leaves and distortion of tips of leaves at the top of the plant at the time of onset of mottling symptoms. These are described in the table as fully invasive, systemic yellow-type strains. Five additional strains produced systemic infections, but did not cause puckering and distortion of young leaves. They are represented as a separate class in the table, as are also 3 that did not produce any systemic symptoms. Yellow-type strains were isolated from 22 separate Turkish tobacco plants previously infected with *masked*-strain virus. Of these, 13 strains proved capable of systemic spread in tobacco, but none showed a high degree of invasiveness. The young leaves of plants infected with yellow-type strains derived from the *masked* virus were either wholly green, or green except for small yellow areas near their tips. Older leaves, although sometimes very yellow, did not lose their natural flatness and were not distorted at their tips. Typical symptoms produced by systemic yellow-mosaic strains derived from *distorting* and *masked* strains are represented in figure 1.

The first two experiments recorded in table 1 were performed simultaneously. In these experiments, 50 tobacco plants were inoculated with the *masked* and a like number with the *distorting* virus. It will be seen from the numbers of attempts to isolate new strains, listed in the table, that more plants infected with the *distorting*-strain (44 altogether) than with the *masked* strain (25 altogether) developed one or more yellow spots during the course of the experiment. About the same number of localized yellow strains, and of systemic, but relatively uninvasive, yellow strains were isolated from each of these sets. The main difference between the sets was the complete failure to obtain fully invasive yellow-mosaic strains from the *masked* strain.

The third and fourth experiments in table 1 were done under conditions of low susceptibility to inoculation. They were performed for the purpose of increasing the total number of isolations of systemic yellow-mosaic strains from the *masked* virus, and were not accompanied by attempts to isolate from *distorting* virus. More failures to secure yellow-type strains occurred, but otherwise the results were comparable with those previously secured, no fully invasive yellow-mosaic strains being obtained from yellow spots on plants infected with *masked* virus.

Other experiments have served to confirm this result. In general, fully invasive yellow-mosaic strains have been derived repeatedly from *dis-*




*Photographed by J. A. Carlile*

FIG. 1. Symptoms produced by typical yellow-mosaic strains derived respectively from *distorting* strain (a, c, e) and *masked* strain (b, d, f) of tobacco-mosaic virus, in *Nicotiana tabacum* (a, b), *Lycopersicon esculentum* Mill. (c, d), and *N. rustica* (e, f).

*torting* virus; but among yellow-mosaic strains derived from *masked* virus there have been none that appeared to be fully invasive.

It has been found that derived strains, if mixed with each other or with original stocks, can be separated by transfer from necrotic primary lesions on such plants as *Nicotiana glutinosa* and *N. langsdorffii*. As a routine technique newly derived strains were in most cases transferred to *N. langsdorffii*, and reisolated from single necrotic primary lesions.

Transfer of strains from necrotic lesions of *Nicotiana langsdorffii* to plants of *N. tabacum* was accomplished by modification of the cork-borer technique previously described by Jensen (4, p. 965). The modification consisted essentially in substituting for the cork borers sharpened tubes made from $\frac{1}{4}$-inch brass tubing, and in their use not only to cut out single necrotic lesions and surrounding tissues, but to crush the tissues, and then to inoculate leaves of healthy plants of *N. tabacum*. During the processes of cutting out lesions and later inoculation, microscope slides of glass were used to support the leaves. Preceding inoculation, the excised leaf material was crushed in a drop of water on the glass slide, by rolling and rubbing the brass tube over it. After use, both instruments were rinsed and sterilized by boiling. The technique is rapid and continuous, allowing transfer from many necrotic lesions without recourse to autoclaving of more bulky instruments, and with no danger of contamination of plants, which are touched only by instruments designated for the particular transfer.

After such transfer to *Nicotiana tabacum* the degrees of yellowing and of invasiveness were again observed in this host species, for each new strain of virus. Virus samples in leaves of *N. tabacum* were preserved in 4-inch lengths of cellophane tubing, with ends closed by twisting. The leaves lost moisture rapidly; when thoroughly air-dried, the closed tube and included leaf fragments were filed in seed envelopes. The imperviousness of cellophane tubing to virus ensured against contamination.

In general the invasiveness of the yellow-mosaic strains derived from *masked*-strain virus did not change during the processes connected with reisolation or storage. The only observed change toward greater invasiveness occurred while a strain recently derived from the *masked* strain was in a tobacco plant. It increased in invasiveness, as shown by subsequent transfer, but comparison with strains derived from both original stocks showed that it could not be classed as fully invasive. It did not cause puckering or yellowing symtoms on young leaves of tobacco plants. Although further variation of strains derived from the *masked* strain might in time disclose full recovery of invasiveness, such recovery has not yet been observed.

Attention was primarily directed to systemic yellow-type strains in these experiments. Whether or not the nonsystemic, *i.e.*, localized, yellow-type strains derived from one source differed as a class from those derived from

the other has not been investigated. A few words with regard to the localized strains may, however, be justifiable. When both localized and systemic yellow strains were isolated from a single yellow spot, only the more invasive form was recorded in the table. Additional nonsystemic yellow-type strains were obtained in some sets of transfers from single yellow spots that also yielded systemic yellow-mosaic strains; these are indicated in the first footnote below the table. All yellow-type strains of tobacco-mosaic virus that were characterized by little or no movement except by slow extension of the primary lesion proved difficult to transfer by the usual rubbing methods. Their transfer was greatly facilitated, however, by sprinkling leaves before inoculation with No. 320 carborundum powder. This was done in accordance with the recommendation of Rawlins and Tompkins (7), who used carborundum powder of a similar size (No. 370) for transfer of tomato spotted-wilt and other viruses.

The usefulness of *Nicotiana rustica* in distinguishing between tobacco-mosaic strains having varied abilities to spread into young tissues of host plants has been pointed out previously (3, p. 849). Since evidence indicated that strains derived from the *masked* strain differed in regard to invasiveness from those derived from the *distorting* strain, *N. rustica* was used to supply additional visual evidence of this difference. Young plants, still in the rosette stage of their development, were inoculated by rubbing. Strains of virus capable of extensive systemic movement in *N. tabacum* caused death of these *N. rustica* plants as a result of numerous necrotic secondary lesions in young leaves and stems. Strains of virus of more limited ability to move systemically caused little or no stunting, and gave few or no secondary necrotic lesions. Photographs showing the effects produced on *N. rustica* by yellow-mosaic strains derived from *distorting* and *masked* strains are represented in figure 1 (e, f).

## DISCUSSION

The *masked* strain of tobacco-mosaic virus, used in these experiments as one source of yellow-mosaic strains, was originally derived from the *distorting* strain of the same virus. Although it shares certain fundamental attributes with the *distorting* strain, it has been shown to differ from this strain in several respects (3). It does not produce yellow patterns in affected leaves. It does not spread into and affect young tissues in plants of *Nicotiana tabacum*. It multiplies at temperatures too high to permit increase of the *distorting* strain.

The inability of the *masked* strain to spread freely in young tissues of such plants as *Nicotiana tabacum* appears to be due to loss of a quality, here called invasiveness, which was inherent in the original *distorting*-strain virus. The characteristic of invasiveness, once lost in the change from the *distort-*

*ing* to the *masked* strain, failed to reappear among derivatives of yellow-mosaic type obtained from the *masked* strain, although it was represented in a majority of yellow-mosaic strains derived directly from the original *distorting*-strain virus. The lack of invasiveness, characteristic of the *masked* strain, was inherited, so to speak, by yellow-mosaic derivatives obtained from it. Thus yellowing and invasiveness may be thought of as independent units, however complex each may prove to be upon further analysis. The question arises whether these and other possible unit differences between strains of tobacco-mosaic virus may not be comparable either to genic differences between parts of chromosomes within plants and animals, or to differences between single allelomorphic genes.

Other differences between the *masked* strain and the *distorting* strain, such as the difference between maximum temperatures for virus increase, and the striking difference in tendency to yellow chlorophyll in invaded tissues of *Nicotiana tabacum,* have not yet been demonstrated to act independently, and may in some instances be due to combined effects of several or many structural changes. Future work may show some of them to be passed on without change to derived strains, and others may fail to be represented in any simple way in derivatives.

The work here reported has demonstrated that from a symptomless strain of virus there may be obtained numerous derivatives capable of causing severe symptoms, at least with regard to interference with the chlorophyll mechanism of the host plant. This change from inability to affect chorophyll to ability to injure it conspicuously is perhaps only of technical interest in the case of tobacco-mosaic virus, severe-symptom strains of which are already world-wide in distribution; but the principles involved may be applicable to consideration of other viruses, in which similar changes may be of practical importance. Such procedures as temporary growth of introduced plants in quarantine, coupled with selection by visual inspection, may be insufficient to prevent importation of vegetatively propagated plants carrying symptomless strains of viruses. After introduction, continued culture may give opportunity for destructive variant strains to arise, and to spread from plant to plant within a country not previously invaded.

Several means may be suggested as possible aids to detection of symptomless strains of viruses. One means of detection depends on the fact that certain plant species serve as dependable indicators of some viruses, by displaying conspicuous and characteristic symptoms irrespective of the strain of the virus used to infect them. A second means of detection involves the use of serum reactions (1, 2). A third means of detection is by observation of occasional occurrence of small irregular-shape yellow spots, similar to those used in the present work as sources of yellow-mosaic variant strains of tobacco-mosaic virus. Isolated yellow spots, in the absence of all other

symptoms, are not usually considered characteristic symptoms of virus diseases, yet appearance of such yellow spots, representing formation of variants, may disclose the presence of virus strains, which of themselves do not visibly affect invaded tissues.

SUMMARY

Yellow-mosaic strains derived from the *masked* strain of tobacco-mosaic virus, although not confined to primary lesions, were found characteristically to lack the high degree of invasiveness possessed by many yellow-mosaic strains derived directly from the *distorting* strain of tobacco-mosaic virus. The uninvasive character that was obtained at the time of isolation of the *masked* strain was thus retained in the systemic yellow-type derivatives of this strain.

Changes to yellow-mosaic type and to invasive type appear to be independent and may represent unit differences in the structure of the virus similar to unit differences in genetic structures of plants and animals.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
PRINCETON, NEW JERSEY

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# THE CALORIFIC VALUE OF DECAYED CORDWOOD

M. T. HILBORN[1]

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## INTRODUCTION

It is well known that fungous decay of wood is a complicated process. Such decay and its rate vary with the kind of fungus, the species and the part of the host, the environmental conditions, and the stage of decay that has been reached by the process. It is also well known that the various changes occurring during the stages of decay include a reduction in the weight of the wood; changes in the chemical composition of the wood; an increase in the water-absorbing capacity of the wood; and an ultimate reduction in the volume of the wood.

A method of measuring fungous decay in wood should be one that measures the progress of these changes. Much of the previous work has been concerned primarily with the changes in specific-gravity values, with changes in chemical composition, or with changes in breaking strength. Since calorific value depends upon the chemical nature of the cell wall and cell contents, as well as upon the amount of these substances present, its determination appears to be applicable in evaluating the fungous decay of wood or other plant materials. Since it measures the amount of wood residue remaining rather than the amount lost it also fulfills the suggestion made in the recent article by Buckman (2).

Lehmann and Scheible (5) enumerate 3 methods of investigating the rate of decay in wood: (1) production of $CO_2$ as an index of the destruction of wood substance, (2) change in specific gravity, and (3) loss in calorific value. All of these methods were employed by them in a controlled laboratory experiment where determinations could be made on inoculated material and on checks. It may be assumed that results obtained in a controlled laboratory experiment are not always applicable to conditions as they exist in the forest or in storage sheds for forest products.

It is the purpose of this paper to apply the method of determining loss in calorific value to a study of decay in 3 species of hardwoods stored in the open in Maine for 4 years under different conditions. The changes measured were the specific gravity when air dried, the calorific value, and the moisture content. On the basis of these measured changes and several

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reasonable assumptions, calculations were made of the practical fuel value of the wood as influenced by species of wood, splitting, part of the tree, type of storage, and length of storage period.

## METHODS AND PROCEDURE

A cord of wood consisting of split and unsplit pieces of three hardwood species, namely: red maple, *Acer rubrum* L.; paper birch, *Betula papyrifera* Marsh.; and beech, *Fagus grandifolia* Ehrh., was purchased in June 1930. This wood had been cut in 4-ft. lengths the preceding winter, and it is assumed that due to the low temperature no measurable amount of decay had taken place during the intervening time.

One half of this cord, consisting of approximately equal numbers of split and unsplit pieces, was piled in the woods at Pushaw Pond, Maine, while the other half was piled in an open field at Orono, Maine, approximately 10 miles away. Throughout this paper these two locations will be referred to as "woods" and "open field" respectively. The piling was done according to approved forestry practice, bark side up on the split pieces. Semi-annually a representative stick (4 feet long) of split and round pieces of each of the species was brought into the laboratory from each location and cut into 6-inch lengths, one of which was chosen as a representative sample for the 4-foot stick and used for all determinations. Laboratory air-dry weights were determined. The specific gravity of the air-dry samples was determined by displacement, after removing the bark from the 6-inch piece. The sample for the calorific value was obtained by drilling a series of holes 1-inch apart with a half-inch auger for the entire length of the 6-inch piece. This was drilled at right angles to the plane of cleavage, and as a result the dry, split edge on the split piece was not used in these samples. The drillings were carefully collected and ground into a fine powder. The moisture content of this sample was determined by drying to constant weight at 105° C. The ash was determined by incinerating a 2-gram sample in a muffle furnace. Aliquot parts, approximately 1 gram each, were taken of this oven-dried powder. These were compacted into pellets and the calorific values were determined in an Emerson Bomb Calorimeter. The data used for further calculations were the averages of two good checks, *i.e.*, within 10 calories variance.

## DATA AND DISCUSSION

The values obtained for specific gravity are shown in figure 1. The writer has no proved explanation to offer for the differences shown between the split and unsplit pieces. When second-growth hardwoods are cut for cordwood in Maine it is the common practice to split the trunk wood and leave the smaller branch wood as round pieces. Paul (6) has found in test-

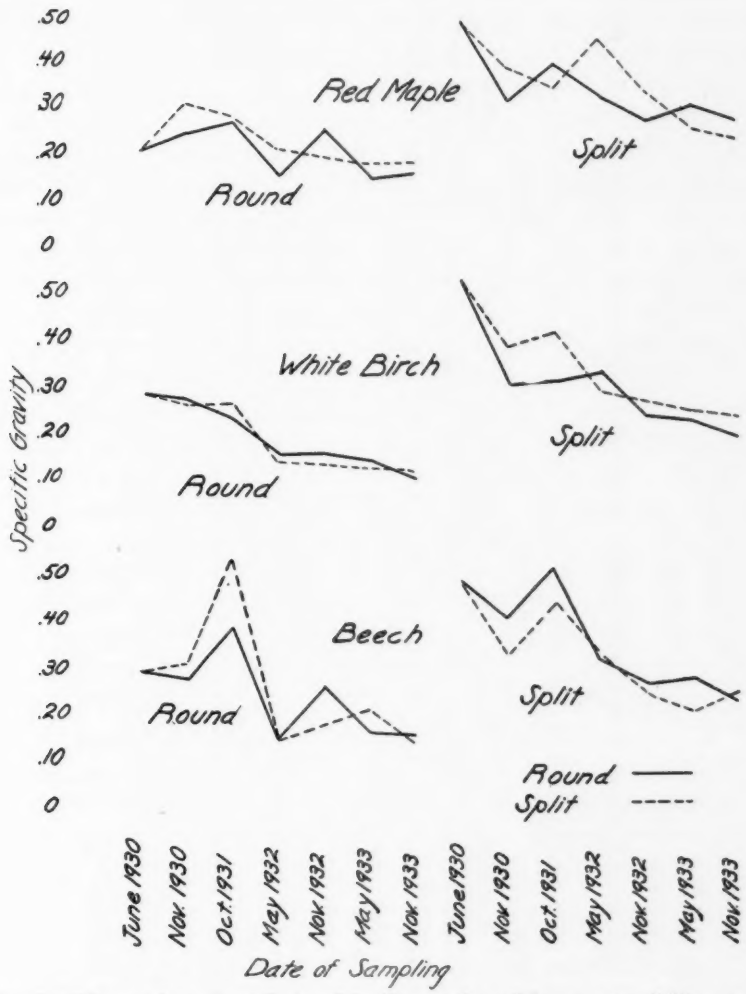


FIG. 1. Specific gravity of wood stored in the woods and in an open field as split and unsplit pieces of 4-foot cordwood.

ing a block of white ash, that in the area near the center of the tree the specific gravity was .65, while in the bark near the sapwood it was only .48. Later, Paul (7) studied the silvicultural aspects of specific gravity and found differences in trees of the same species growing only a rod apart. Similar factors may enter into the causation of the variable values given here.

The data presented in table 1, giving the B.T.U. values per pound, suggest that possibly the fungi have removed some components that may detract from or at least not contribute toward the heat value. By comparing figure

TABLE 1.—*Calorific value of wood stored in the woods and in an open field as split and unsplit pieces of cordwood. B.T.U. per pound of wood*[a]

| Date | Red maple | | | | White birch | | | | Beech | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Woods | | Open field | | Woods | | Open field | | Woods | | Open field | |
| | Round | Split | Round | Split | Round | Split | Round | Split | Round | Split | Round | Split |
| June 1930 | 8572.1 | 8551.3 | 8572.1 | 8551.3 | 8432.9 | 8389.7 | 8432.9 | 8389.7 | 8956.0 | 9080.5 | 8956.0 | 9080.5 |
| Nov. 1930 | 8743.8 | 8193.7 | 8743.8 | 8193.7 | 8349.6 | 7689.3 | 8349.6 | 7689.3 | 8743.8 | 8780.4 | 8743.8 | 8780.4 |
| May 1931[b] | ..... | ..... | 8610.4 | 8093.1 | ..... | ..... | 8378.2 | 7843.6 | ..... | ..... | 8845.1 | 8919.8 |
| Oct. 1931 | 8750.3 | 8701.2 | 8746.9 | 8436.3 | 8012.9 | 7935.2 | 8136.7 | 7768.5 | 8873.4 | 8426.5 | 8916.5 | 7983.1 |
| May 1932 | 7772.1 | 8258.7 | 8315.2 | 7783.5 | 7540.5 | 7711.3 | 7651.4 | 7658.4 | 7800.2 | 8112.3 | 7836.1 | 7924.7 |
| Nov. 1932 | 8503.7 | 7637.1 | 7948.3 | 8043.1 | 7697.6 | 7673.0 | 7538.1 | 7646.1 | 8572.7 | 7758.3 | 7893.2 | 7793.5 |
| May 1933 | 7546.1 | 7552.4 | 7648.9 | 7362.6 | 7640.8 | 7581.3 | 7371.2 | 7599.0 | 7753.6 | 7625.1 | 7813.9 | 7862.0 |
| Nov. 1933 | 7369.4 | 7485.4 | 7428.6 | 7016.8 | 7101.8 | 7410.3 | 7169.2 | 7458.7 | 7720.6 | 7784.3 | 7700.5 | 7741.6 |

[a] Converted from calories per gram by multiplying by the factor 1.8.

[b] No samples were taken from the woods on this date.

1 with table 1 it is shown that the specific gravity decreased considerably faster than the fuel value on a per pound basis. This is contrary to previous opinions. Lehmann and Scheible (5) state . . . "Heretofore it has been generally held that losses in heat value which a given mass of wood may undergo through the action of fungi correspond to its losses in dry weight. . . ." The data they present do not support this view, so far as concerns pine sawdust. The results of this investigation likewise do not agree with the above cited opinion.

In order to interpret the results in a form applicable to general use, it was found necessary to determine the relative fuel value per cord. The first step was to learn the volume of wood per cord. This was done by first taking the approximate size of the sticks of each species and adopting the values for the respective sizes from a volume table such as given by Chapman (4, p. 131). It was then necessary to determine the pounds per cubic foot. This was done by taking the figure 62.5, the weight of 1 cu. ft. of water at 4° C., and multiplying it by the specific gravity. This, of course, disregards temperature, but the assumption was necessary in order to secure data applicable to the case. It was then possible to calculate the total absolute number of B.T.U. per cord. In all probability the method of sampling accounts for some of the variation in the values derived. This was partially compensated for by interpolation curves. The B.T.U. values per cord were converted to a percentage basis with the first or undecayed sample taken as 100 for each series. The results are shown in table 2.

A comparison of the figures given in table 2 for the samples from the woods in November, 1933, and those from the open field show that those in the woods have, with the exception of the split red maple and the round beech, lost more than the corresponding samples in the open field. The irregularity in the values given for these two series may be due to variation in the sampling, which was not smoothed out by the interpolation curves. Although the final values for the samples of split pieces in only half the cases are lower than the values for the corresponding samples of round pieces, in most of the pairings the values are lower for the samples of the split pieces. As shown in table 1, this relationship is maintained for the B.T.U. values per pound. Thus it is apparent that the samples of some of the split pieces have deteriorated more rapidly than the samples of the round pieces. Possibly the protective coating of bark on the round samples may have influenced this; although, as a rule, splitting is considered to permit rapid drying with the inference that deterioration through decay would be less. In that the dry and apparently sound part of the split pieces was intentionally avoided in the sampling, the comparison, in all probability, was not influenced by splitting of the sticks.

TABLE 2.—*Relative calorific value in absolute B.T.U. per cord for wood stored in the woods and in an open field as split and round 4-foot pieces of cordwood.* (Based upon an interpolation curve)

| Date | Red maple | | | | White birch | | | | Beech | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Woods | | Open field | | Woods | | Open field | | Woods | | Open field | |
| | Round | Split | Round | Split | Round | Split | Round | Split | Round | Split | Round | Split |
| June 1930 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Nov. 1930 | 93 | 92 | 96 | 86 | 91 | 80 | 98 | 90 | 95 | 95 | 96 | 82 |
| May 1931 | 85 | 84 | 86 | 71 | 85 | 69 | 91 | 81 | 90 | 91 | 92 | 70 |
| Oct. 1931 | 81 | 70 | 83 | 65 | 77 | 54 | 86 | 73 | 83 | 85 | 89 | 67 |
| May 1932 | 75 | 68 | 82 | 55 | 66 | 57 | 77 | 64 | 79 | 75 | 80 | 64 |
| Nov. 1932 | 70 | 60 | 81 | 49 | 59 | 41 | 62 | 53 | 70 | 65 | 75 | 61 |
| May 1933 | 67 | 54 | 80 | 44 | 56 | 39 | 50 | 48 | 60 | 56 | 65 | 58 |
| Nov. 1933 | 66 | 48 | 76 | 40 | 32 | 34 | 38 | 39 | 47 | 44 | 46 | 46 |

As is shown in table 2, the rate of deterioration was about equal in both locations. The values are, however, somewhat lower in the woods than in the field. A comparison of the values for the round *vs.* split samples shows that the rate of deterioration is slightly less in the round form than when the wood has been split. The round red maple from the field shows a striking divergence from the split red maple in the same location.

A comparison of the values for each species shows the greatest loss near the end of the experiment for the round white birch. This is well illustrated in the values for the round samples from the woods. The values for the beech show about the same tendency as the white birch for the round samples; the values for the split samples, however, are erratic, especially at the beginning of the experiment.

Lehmann and Scheible (5) investigated the loss in calorific value of pine sawdust when inoculated with various fungi. They recorded a maximum loss of 42.9 per cent in the heating value of the sawdust after a 6-month period of incubation with *Armillaria mellea*. Other fungi gave lower readings. *Daedalea quercina* caused a loss of only 8.1 per cent. In the 3 species of hardwoods reported here no specific attempt was made to determine what fungi were affecting each sample of wood. Fruiting bodies of the following species were collected from the wood in both locations: *Polyporus hirsutus* (Wulf.) Fr., *P. pargamenus* Fr., *Panus stipticus* Bull., *Stereum purpureum* Pers., *Thelephora* sp., and *Daldinia* sp. In all probability other fungi were present, but were not in fruit. Vanin and Esupoff (9) also investigated the effect of decay on the calorific value of wood. They give a very good résumé of previous work done in this field in Russia. Their results show that wood loses from 21.4 to 72.3 per cent of its fuel value through decay. Unfortunately, the time interval was not given in the translation of their paper, and it, therefore, is impossible to compare their results with those reported here.

The values given in table 2 represent the absolute B.T.U. per cord, and do not take into consideration the amount of water present in the wood. As decay advances the moisture content increases considerably. Campbell (3) had stated that to heat a pound of water from the ordinary temperature of the room to the boiling point, evaporate it, and heat the steam to the temperature of the flue gases requires approximately 1200 B.T.U. Record (8) gives a similar figure. Consequently, the increase in moisture content reduces proportionally the practically useful calorific value of wood containing a given number of heat units.

When the total pounds of water in the wood is computed per cord and a correction applied, the useful B.T.U. values per cord can be calculated. This varies somewhat from the absolute B.T.U. values, especially in the 1932 and 1933 samples. Betts (1) gives a formula for comparing the price to

TABLE 3.—*Relative value per cord of the respective species stored in the woods and in an open field as split and round pieces of 4-foot cordwood.* (Based upon interpolation curves)

| Date | Red maple | | | | White birch | | | | Beech | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Woods | | Open field | | Woods | | Open field | | Woods | | Open field | |
| | Round | Split | Round | Split | Round | Split | Round | Split | Round | Split | Round | Split |
| June 1930 | $8.00 | $8.00 | $8.00 | $8.00 | $8.00 | $8.00 | $8.00 | $8.00 | $8.00 | $8.00 | $8.00 | $8.00 |
| Nov. 1930 | 7.10 | 6.60 | 7.80 | 5.50 | 6.65 | 6.45 | 7.48 | 6.25 | 6.90 | 6.93 | 7.70 | 6.38 |
| May 1931 | 6.20 | 5.28 | 7.55 | 4.10 | 5.35 | 4.95 | 6.60 | 4.65 | 5.75 | 5.85 | 7.05 | 4.90 |
| Oct. 1931 | 5.40 | 4.15 | 7.25 | 3.40 | 4.10 | 3.60 | 5.30 | 3.40 | 4.68 | 4.68 | 5.80 | 3.70 |
| May 1932 | 4.70 | 3.25 | 6.90 | 3.20 | 3.00 | 2.55 | 3.68 | 2.42 | 3.72 | 3.75 | 4.25 | 2.75 |
| Nov. 1932 | 4.18 | 2.60 | 6.45 | 2.90 | 2.10 | 1.73 | 1.90 | 1.71 | 2.95 | 2.20 | 3.10 | 2.10 |
| May 1933 | 3.70 | 2.20 | 5.90 | 2.30 | 1.35 | 1.20 | 1.20 | 1.36 | 2.33 | 1.70 | 2.35 | 1.75 |
| Nov. 1933 | 3.64 | 2.13 | 5.13 | 1.43 | .85 | .94 | 1.06 | 1.32 | 1.87 | 1.66 | 1.99 | 1.73 |

be paid for wood in order to make it as economical to use as coal. This may be applied to the actual B.T.U. produced per cord in order to determine comparable values at any succeeding date after cutting in order to obtain a comparable number of heating units. Assuming the useful B.T.U. in the original samples as being worth $8.00 per cord, and correcting for changes in water content and absolute B.T.U. in subsequent samples, the values secured are given in table 3.

As shown in table 3, the cordwood stored in the woods is appreciably lower in value than that stored in the open field. If this wood had been stored in the well-ventilated shed, its value would not have changed appreciably, inasmuch as the moisture content of the samples collected in June, 1930, was less than 30 per cent, which is considered near the minimum for fungal action. The loss is especially significant if we consider the round white birch stored in the woods. After 3 years' storage its value becomes only $.85 per cord, while, under proper storage conditions, its value would still be approximately $8.00.

## SUMMARY

Assuming that all deterioration observed was caused by fungous decay, the effect of such decay on specific gravity and calorific value was measured in split and unsplit wood of 3 species, red maple, *Acer rubrum* L.; paper birch, *Betula papyrifera* Marsh.; and beech *Fagus grandifolia* Ehrh., in 2 types of situations over a period of 4 years.

Specific gravity of the wood when air-dried was found to be higher in split wood, presumably from trunks, than unsplit wood, presumably from branches.

As the specific gravity decreased, the calorific value of the wood, air-dried, also decreased somewhat, indicating a change in chemical composition.

The calorific value decreased somewhat faster in the woods than in an open field, faster in the split than in the unsplit form, and respectively more rapidly for the maple, beech, and birch in the round form.

Correcting the calorific value for water content, which increased with decay, the net or practically useful fuel value was found to decrease in 4 years as much as 89.4 per cent for the round white birch from the woods, and as little as 35.9 per cent for the round red maple from the open field. The decrease was as much as 50 per cent in less than 2 years in some of the birch wood.

UNIVERSITY OF MAINE,
ORONO, MAINE.

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# APPEARANCE OF FOMES IGNIARIUS IN CULTURE

HENRY HOPP

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Wood-destroying fungi commonly produce a rather constant type of mycelial growth in culture. *Fomes igniarius* (L.) Gill., one of the most destructive of the xylophilous fungi, is peculiar in that it does not follow this general rule. Previous workers report that, with the exception of those obtained from species of Populus, sporophore-tissue cultures of this fungus isolated from different hosts are similar in appearance. The marked differences in appearance between the cultures obtained from *Populus tremuloides* Michx. and those obtained from *Alnus* sp. led Long and Harsch[1] to the belief that two different fungous species were concerned. Fritz[2] noted these differences but minimized their diagnostic value, since transfers from some of her cultures obtained from Populus did produce mycelium similar to that isolated from other hosts.

In the investigations cited the cultures were all started from sporophore tissue. In the study herein reported, the cultures were obtained from decayed wood. Three trees of *Populus tremuloides* infected with *Fomes igniarius* were used. One hundred and twenty small pieces of wood were taken from various places in the decayed trees and placed on Bacto malt agar in 31 Petri dishes. From these plantings, only 50 fungous cultures were obtained. A survey of the cultures showed that 2 types of mycelium were present. Forty-one cultures were of the type that, according to the literature, is obtained only from Populus. It was characterized by deep staining of the agar, and in color approximated mummy brown[3]. Surface mycelium was exceedingly sparse, and aerial mycelium was almost or entirely absent. This type of culture is referred to in this paper as the staining type. Nine cultures were of the type that, according to previous workers, is obtained only from sources other than Populus. The latter kind of culture, hereafter referred to as the bleaching type, was characterized by a more-or-less evident bleaching of the agar and by profuse aerial mycelium approximating antimony yellow to Dresden brown. In 5 of the 9 cultures, the agar was stained during the early growth of the mycelium, but was bleached during subsequent growth, while in the other 4 cultures, it was unstained from the beginning.

These results suggested that an effort to determine the cause for the 2 types of cultures might be worthwhile. To this end an attempt was made

[1] Long, W. H., and R. M. Harsch. Pure cultures of wood-rotting fungi on artificial media. Jour. Agr. Res. [U. S.] **12**: 33–82. 1918.

[2] Fritz, C. W. Cultural criteria for the distinction of wood-destroying fungi. Roy. Soc. Canada, Proc. and Trans. III, **17** (Sect. V): 191–288. 1923.

[3] The color nomenclature in this paper is that used by Ridgway, R. Color standards and color nomenclature. Washington, D. C. 1912.

to correlate the number of cultures of each type with (a) the individual host, (b) the zone of decay, and (c) the level along the longitudinal axis of the tree from which the inocula had been taken.

The decayed area was differentiated into several zones that formed concentrically arranged vertical cylinders within the stem of the tree. As viewed on a cross-section surface, the centermost cylinder was a zone of yellowish advanced decay. This was enclosed within a black border line, a narrow band encircling the advanced decay and separating it from the noninfected wood or from a dark zone of brown incipient decay. Within the advanced decay area, black lines were sometimes found. Their positions indicated that they were formerly border lines that persisted following the centrifugal extension of the advanced decay. Inocula were taken from the incipient decay, the black border line, the advanced decay, and the black lines contained within the advanced decay.

Inocula also were obtained at 4 levels along the longitudinal axis in the decayed portion of the trees. The different levels were (a) *above* the upper end of the black border line; (b) directly *at* the upper end of the black border line; (c) a short distance *below*, but within one meter, of the upper end of the black border line; and (d) near the *base* of the tree.

The data in table 1 indicate that the appearance of the cultures was independent of the source of the inocula. The type of culture was definitely not related either to the level in the tree or to the zone of decay from which the inocula were obtained. The production of only the staining type of culture from tree number 2 would suggest a host influence, except that inocula from trees number 1 and 3 gave both types of cultures.

TABLE 1.—*The number of staining and bleaching cultures obtained from Populus tremuloides decayed by Fomes igniarius*

| Source of inoculum | Type of culture obtained | |
|---|---|---|
| | Staining | Bleaching |
| Tree number: | | |
| 1 | 13 | 4 |
| 2 | 19 | 0 |
| 3 | 9 | 5 |
| Zone of decay: | | |
| Incipient decay | 0 | 1 |
| Black border line | 15 | 2 |
| Advanced decay | 23 | 6 |
| Advanced decay and black lines therein | 2 | 0 |
| Black lines within advanced decay | 1 | 0 |
| Level in the tree: | | |
| Above upper end of black border line | 18 | 5 |
| At upper end of black border line | 2 | 0 |
| Below upper end of black border line | 14 | 3 |
| Near base of tree | 7 | 1 |

It was observed that, regardless of their origin, inocula usually produced the same type of culture when planted in the same Petri dish. Eighteen of the 31 Petri dishes contained more than one culture each. Both bleaching and staining types occurred in only 2 of these. Each of the other Petri dishes contained cultures of one type or the other, but not of both types. In 3 cases, duplicate inocula taken from within less than 2 centimeters of each other in the advanced decay produced different types of cultures when they were planted on agar in separate Petri dishes.

Thirty-two transplantings in 8 Petri dishes were made from 2 bleaching and 2 staining cultures, so that within a single Petri dish 2 inocula from each type of culture were grown. The subcultures were of the same 2 types, and, interestingly enough, inocula taken from the staining cultures produced both staining and bleaching subcultures. The cultures obtained in one Petri dish were generally similar in appearance irrespective of their source, although inocula taken from the same source, but grown in separate Petri dishes, produced in 6 cases subcultures of different types. Multiplicate transfers from 10 additional cultures confirmed the observation that growth of both types can be secured from the same mycelium when it is placed on agar in different Petri dishes.

It was recognized that tissue cultures obtained from decayed wood might contain more than an individual fungus. In order to isolate individual organisms, single hyphal tips, cut from mycelium growing on nonnutrient agar, were cultured in separate Petri dishes. Multiplicate transfers from these cultures showed that mycelium originating from a single hyphal tip can produce both staining and bleaching types of growth. Single-spore cultures were then studied in order to determine whether they would give variations in type when subcultured. Accordingly, 13 spores from a sporophore of *Fomes igniarius* produced on *Populus tremuloides* were isolated and placed on malt agar. Among the resulting cultures were staining and bleaching types; and multiplicate subcultures from each of these were also of both types.

These data indicate that *Fomes igniarius* isolated from wood of *Populus tremuloides* may produce 2 types of mycelial growth on malt agar. Homothallic cultures obtained from mycelium growing in wood and from spores also produced both types of growth. The type of mycelial growth in cultures obtained from Populus has little to do with the source of the inoculum but is related to some other factor yet unknown. The conditions of the culturing, however, might be of some importance in determining the type of resultant culture. No indication was observed of a genetic difference in the mycelium found in the bleaching and staining cultures isolated from Populus.

DEPARTMENT OF FOREST BOTANY AND PATHOLOGY,
NEW YORK STATE COLLEGE OF FORESTRY,
SYRACUSE, NEW YORK.

## PHYTOPATHOLOGICAL NOTES

*Spotted Wilt of Garden Pea.*—In October, 1934, certain pea plants (*Pisum sativum* L.) in one of the University of Wisconsin greenhouses were affected by a streaking of stem and a spotted brown necrosis of leaves that often resulted in their premature death. Inoculation of expressed juice from these disease plants into tobacco, tomato, aster, nasturtium, *Nicotiana glutinosa* L., *Datura stramonium* L., and *Emilia sagittata* DC., produced symptoms identical with those associated with the virus of spotted wilt of tomato. In 12 out of 18 Yellow Admiral pea plants inoculated by rubbing with juice from spotted-wilt-infected Calla lily leaves (virus from M. W. Gardner, California) and in 4 out of 16 plants inoculated by the tissue-insertion method of Stubbs,[1] symptoms developed, identical with the naturally occurring streak. Ten noninoculated plants remained healthy. Carborundum powder, used as an abrasive, greatly facilitated infection. Pea plants of varying stages of growth were infected by the rubbing method, but the highest percentage of infection occurred on seedlings inoculated soon after emergence, and before the trifoliate leaves had unfolded. From 105 Yellow Admiral, Alaska, and Alderman plants, inoculated by the rubbing method, 33 showed typical symptoms and the 3 varieties appeared to be about equally susceptible.

Infective thrips (*Thrips tabaci* Lind.) were transferred to healthy peas in one series from nasturtiums infected with the pea-streak virus, and in another series from nasturtiums infected with the spotted-wilt virus. Symptoms developing in both series appeared to be identical and typical of the original pea-streak disease.

Of 192 plants so treated 43 showed symptoms. Results were positive in each of 6 attempts to recover the virus from peas thus inoculated. When infective thrips were caged on 27 pea blossoms, 17 set pods and symptoms appeared on 6, from which the virus was readily recovered.

Under these experimental conditions the first symptoms usually appear 7 to 20 days after inoculation as necrotic streaks on the stem and as spot or vein necrosis on young leaves. Frequently, however, the initial symptom appears as a distinct necrotic spot on the artificially inoculated leaves, or in association with thrip-feeding injury when the virus is transmitted by that vector. The stem streak is purplish or bluish brown and may extend only a little beyond the point of inception or over the entire length of the stem. A histological examination of infected stems showed general necrosis of parenchymatous cells, including the phloem. Unilateral development of affected parts is frequent. Occasionally a mottled pattern develops on leaves

[1] Stubbs, M. W. Viroses of the garden pea, *Pisum sativum*. (Abs.) Phytopath. 26: 108–109. 1936.

infected when young. Local infections of immature pods may appear as small, circular necrotic spots associated with the thrips-feeding marks, and necrosis may later extend into irregular, wavy, concentric patterns over most of the surface. Systemic infection may result also in necrotic or concentric patterns on the pods; however, if these are systemically or locally infected when very young, they often become stunted, distorted, and collapsed. The seeds occasionally show necrotic spots or patterns (Fig. 1).

FIG. 1. Symptoms of spotted wilt on pea. A. Necrotic lesions on naturally infected pea seed. B–C. Pods, naturally infected in the field, showing mild and severe symptoms. D. Artificially inoculated pea plant showing severe necrosis of growing point, stem, and leaflets and unilateral development of affected parts.

Linford noted a streak of peas, widely distributed in the United States, in 1928.[2] He later produced similar symptoms by transfer of thrips to pea from *Emilia sagittata* affected with pineapple yellow spot,[3] and found pea-streak plants near Honolulu, Hawaii, naturally infected with this virus. He suggested that this or a related virus caused pea streak in the United States. Stubbs produced a streak symptom in pea by inoculation with tobacco ring-spot virus, and Zaumeyer and Wade[4, 5] produced a streak in pea by inoculation with viruses from white sweet clover, sweet clover, and alfalfa. The demonstration that the tomato spotted-wilt virus causes a streak of pea is of peculiar interest inasmuch as the virus has much in common with that of pineapple yellow spot in the way of insect vector, incubation period, and almost identical symptoms on *Emilia sagittata*.[6] In view of the fact that the spotted-wilt virus is widely distributed it is undoubtedly commonly a causal factor in pea streak. Symptoms similar to those described in the greenhouse were observed in the field at Madison, Wisconsin, in the summer of 1935 on pea plants artificially inoculated with the spotted-wilt virus. An epidemic of pea streak, which developed in two widely separated pea nurseries at Madison in the same year was due in part to spotted wilt, inasmuch as the virus was recovered from badly diseased plants.—OTIS C. WHIPPLE, University of Wisconsin, Madison, Wis.

*Injury to Greenhouse Tomatoes as a Result of a Combined Infection with the Viruses Causing Tomato and Cucumber Mosaic.*—During the spring of 1935, two cases came to the writers' attention in which greenhouse tomatoes in Ohio and Colorado were severely damaged by a virosis caused by a combined infection with ordinary tomato mosaic (*tobacco virus* 1, Johnson) and cucumber mosaic (*cucumber virus* 1, Doolittle). The tomato is known to be susceptible to both, either singly or combined, but the combined infection, apparently, has rarely assumed economic importance. In both cases, however, the losses were estimated at some thousands of dollars and it is evident that such infection occasionally may prove a definite menace to the crop. The Colorado and Ohio specimens were alike in appearance, and the writers also observed the disease as it occurred in the Ohio greenhouse.

[2] Linford, M. B. Pea diseases in the United States in 1928. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Reptr. Sup. 67. 1929. (Mimeographed.)

[3] Linford, M. B. Streak, a virus disease of peas transmitted by *Thrips tabaci*. (Abs.) Phytopath. 21: 999. 1931.

[4] Zaumeyer, W. J., and B. L. Wade. Mosaic diseases affecting different legumes in relation to beans and pea. Phytopath. 23: 562–564. 1933.

[5] Zaumeyer, W. J., and B. L. Wade. A pea streak caused by alfalfa mosaic. (Abs.) Phytopath. 26: 114. 1936.

[6] Linford, M. B. Transmission of the pineapple yellow-spot virus by *Thrips tabaci*. Phytopath. 22: 301–324. 1932.

In this latter case the tomatoes had been planted in November and, when observed on May 30, were abnormally short (3–4 feet) and compact. The leaves just below the growing point had a peculiar upright, bushy habit of growth that was due partly to very short internodes and partly to an abnormally twisted and erect growth of the petioles (Fig. 1). The young leaflets

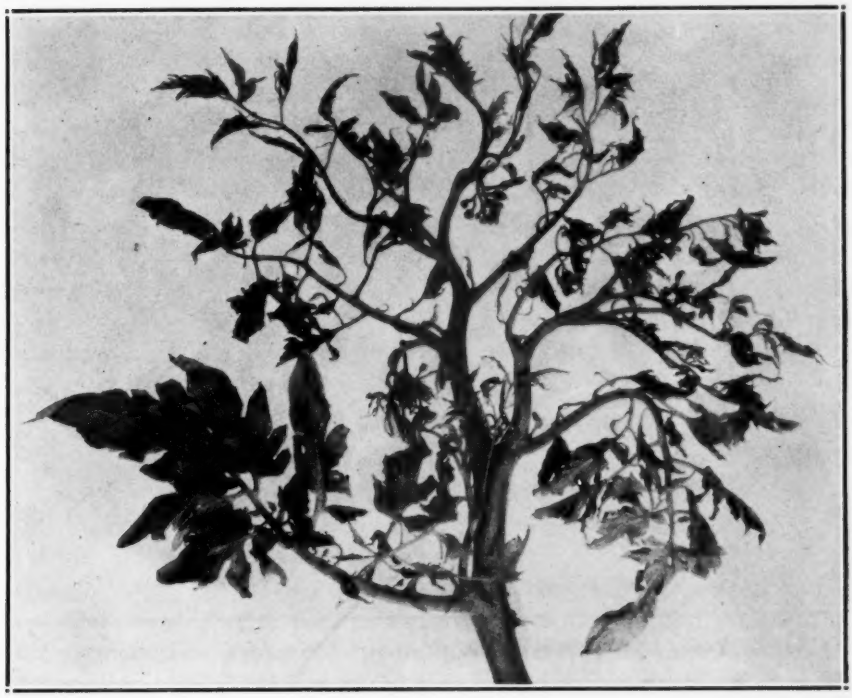

FIG. 1. Upper portion of greenhouse tomato plant showing malformations of foliage and peculiar habit of growth due to combined infection with the viruses of cucumber and tomato mosaic.

were curled and distorted and occasionally showed the filiform "shoestring" malformations that often occur on tomatoes infected with cucumber mosaic. These leaflets were thick and showed a pronounced yellowing of the veins. The older leaflets generally were rolled sharply upward at the margins and carried a mild, yellow-green mottling similar to that of ordinary tomato mosaic. Many of the leaves, however, were of a peculiar greenish purple, particularly along the veins and on the under surface of the leaf. Some leaves also showed large, yellow spots of a bleached appearance accompanied by a russet-color necrosis bordering the larger veins. This necrosis, however, was not sufficiently severe to cause serious injury to the leaf. The blossoms were commonly malformed and abortive; but the plants had produced some

fruit, which was deeply ridged and, when small, showed a characteristic pointed protuberance at the blossom end (Fig. 2). These symptoms are not characteristic of either of the viruses alone and were new to the writers.

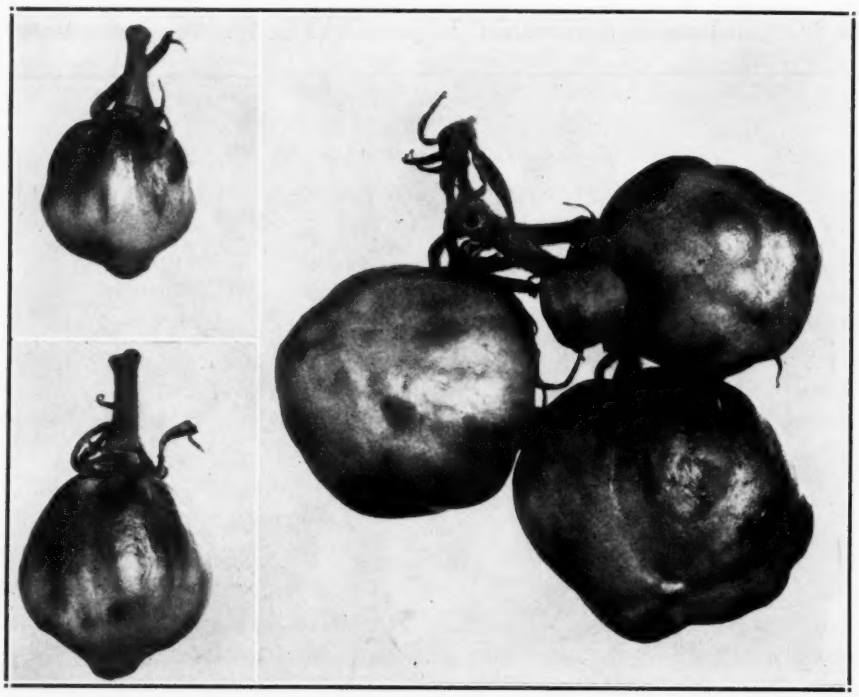

FIG. 2. Young tomato fruits showing irregular, ridged growth and pointed protuberance at blossom-end caused by a combined infection with the viruses of cucumber and tomato mosaic.

Inoculations were made by the senior writer to tomato, cucumber, and tobacco, using diseased tomato material from both Colorado and Ohio. The inoculated tomato plants developed the peculiar leaf color and necrosis already described, and the later growth showed leaf malformations similar to those noted in the greenhouse. Since the cucumber is not susceptible to tobacco mosaic, the cucumber virus was readily separated from the accompanying tobacco virus by direct inoculations to cucumber. On both cucumber and tobacco the cucumber mosaic virus produced symptoms characteristic of cucumber virus 1. The tobacco mosaic virus was separated from the cucumber virus by heating the extracted juices of plants carrying both viruses to 82° C. On tobacco and tomato, the remaining virus appeared identical with tobacco virus 1. Inoculations to *Datura stramonium* indicated that the common latent mosaic of potato was not a factor in the disease in question.

Owing to its rapid dissemination by pruning and handling, tomato mosaic is so prevalent on greenhouse tomatoes that the introduction of another virus (such as cucumber mosaic) is almost certain to result in combined infection. Cucumber mosaic, however, is less readily transmitted to tomatoes by mechanical means, and aphids apparently are responsible for most of its secondary dissemination. This probably accounts for the rarity of losses from the combined infection, since aphids are effectively controlled in most tomato greenhouses. In Ohio, the cucumber mosaic infection was traced to a near-by field of muskmelon, and, in Colorado, it apparently originated on cucumbers in another section of the greenhouse. In both instances aphids apparently were present in considerable numbers. In the fall of 1935, the combined infection appeared on 2 plants in the greenhouse at Wooster, Ohio, evidently as a result of aphids carrying the cucumber virus from cucumbers in the field. A few infected plants also were noted in another Ohio greenhouse, but in both instances periodic fumigation was practiced and there was no secondary spread of the disease.—S. P. DOOLITTLE, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture and L. J. ALEXANDER, Department of Botany and Plant Pathology, Ohio Agricultural Experiment Station, Wooster, Ohio.

*A Method of Separating the Teliospores of Cronartium Ribicola.*—The teliospores of *Cronartium ribicola* Fischer, the blister rust of five-needle white pines, are borne in slender hair-like columns (Fig. 1, A) on the under surface of the leaves of various Ribes species. Studies now in progress, involving a quantitative determination of the teliospore-production capacities of several Ribes species, necessitated finding a method of separating the spores from the telial columns, in order that rapid and accurate counts of spores per column could be secured. The primary obstacle confronting simple separation of the spores is a matrix that binds the teliospores (Fig. 1, B) firmly together in compact columns. So inert is this matrix that ordinary hydrolytic agents do not render it soluble if used in concentrations sufficiently dilute not to be corrosive to the teliospores. Mechanical maceration following hydrolysis merely resulted in breaking the spores transversely without separating them from the matrix. The ideal reagent sought was one that would dissolve out the matrix but leave the column intact to facilitate mounting singly on a slide, permitting complete separation of the teliospores (Fig. 1, C) by rolling the column between slide and cover slip. It finally was discovered that a 1-Normal nitric acid solution, if properly manipulated, had all the desired features. The solubility of the matrix in nitric acid indicates that it possibly contains some pectic substance. Confirmatory evidence of this has been obtained when the solution, in which a

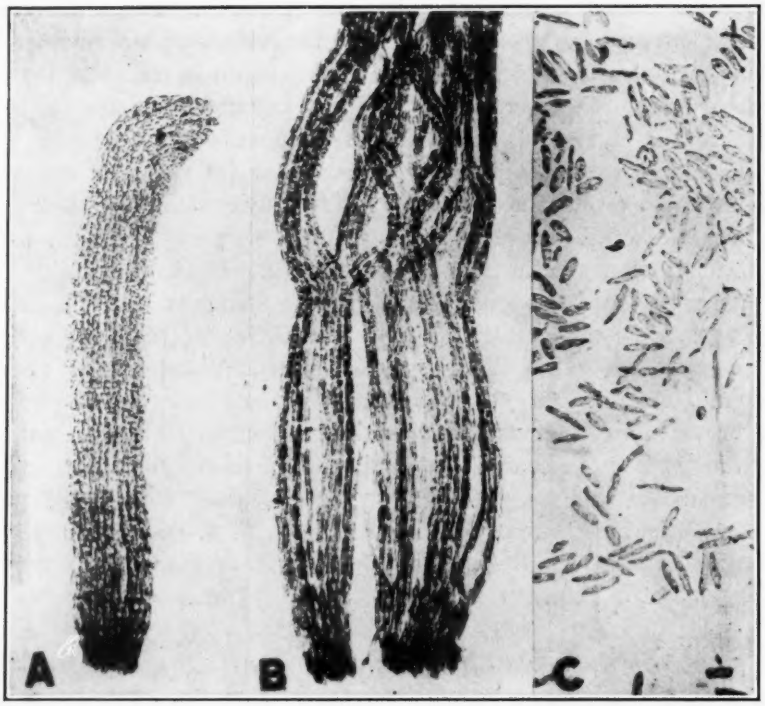


FIG. 1. A. Entire mature column of *Cronartium ribicola* after nitric acid treatment The general appearance of the column is slightly affected by the treatment. B. Longitudinal splitting of the teliospore chains. C. Complete separation of the teliospores. All × 90.

large volume of telial columns had been treated, was further treated with concentrated nitric acid and then yielded crystals of mucic acid.

The following procedure has given consistent results in routine laboratory practice of separating teliospores. Fragments of fresh or dried ribes leaves bearing telial infection are placed in a receptacle and covered with 1-Normal nitric acid. The mixture is boiled vigorously over a flame until the columns begin to become detached from the leaf surface and take on a bleached appearance. Cold water is quickly added to halt the reaction. More columns are removed by stirring with a glass rod, also used to remove the leaf fragments from the solution. The columns settle rapidly out of suspension, permitting the water to be decanted off with no great loss of material. Four or 5 additional changes of water suffice to wash out the remaining acid. The use of alkalies to neutralize to acid has no advantage and usually results in discolored spores. Nearly all of the remaining water can be removed from the columns by transferring to a small vial and draw-

ing it out with a pipette. The columns are then ready for mounting in glycerine or glycerine jelly or they can be kept in glycerine to which some eosine or erythrosin has been added.

If correctly treated the entire mature column should appear as illustrated in figure 1, A, and under slight pressure should split as shown in figure 1, B. Further pressure and movement of the cover slip will break up the chains of spores and complete the separation (Fig. 1, C). The method has been tried and used with success on *C. ribicola* from 10 Ribes species native to western United States and on *C. occidentale* Hedgcock, Bethel and Hunt, the pinyon blister rust, from 2 species of ribes native to its range.—R. K. PIERSON.

DIVISION OF FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE,
PORTLAND, OREGON.

(In cooperation with Emergency Conservation Work and the School of Forestry, University of Idaho.)

*A Preliminary Note on Sexuality in Ceratostomella ulmi.*—In 1932 Buisman[1] reported that the Dutch elm disease fungus *Graphium ulmi* had an ascomycetous stage and was heterothallic. She named it *Ceratostomella ulmi.* Its perithecia have not been found on diseased trees in this country, but their importance in the life history suggested an investigation of sexuality in the fungus and of the distribution of races of the fungus in the United States.

Mass isolates secured from the Dutch Elm Disease Laboratory at Morristown, New Jersey, and approximately 2,000 monoconidial isolates from them were tested for formation of perithecia by growing them singly and in combinations on sterilized elm twigs. All monospore isolations were made with a micromanipulator or microloop.[2]

Monoconidial isolates did not form perithecia, whereas crosses of some of them from different mass isolates did. In a few cases crosses of monoconidial isolates from the same mass isolate also produced perithecia. In such cases it was later determined that both plus and minus races were present in the original isolate.

Monoascospore isolates from perithecia resulting from pairing monoconidial isolates have repeatedly produced the Graphium stage in culture. An American elm, inoculated under quarantine conditions with a suspension

[1] Buisman, Christine. *Ceratostomella ulmi.* De geschlachtelijke vorm van *Graphium ulmi* Schwarz. Tijdschr. Plantenz. 38: 1–5. 1932.

[2] Dunn, M. S. The microloop. A rapid method for isolating single spores. Phytopath. 14: 338–341. 1924.

made from a monoascospore isolate, developed typical symptoms of the Dutch elm disease after two weeks. The Graphium stage was recovered from the tree.

Monoascospore isolates did not form perithecia, but, when paired, perithecia developed in some crosses within 9 to 42 days. Some crosses produced abundant perithecia; others, only a few. The results of crosses among 14 monoascospore isolates are given in table 1.

The perithecia secured by the writer agree morphologically with those described by Buisman. She, apparently, observed no asci. The writer observed asci in different stages of development by killing the material in 75 per cent chrom-acetic acid and mounting in lacto-phenol.[3] The asci are hyaline, clavate, tapering sharply to the point of attachment, and measure 8.75–15μ by 3.1–6.8μ averaging 10.9μ by 5.36μ at the widest portion. The number of spores in an ascus could not readily be determined; in 2 cases it was thought that 8 were observed.

Isolates from diseased trees in the United States were grown in combination with races of *Ceratostomella ulmi* designated as plus or minus, on the basis of their consistent reaction in previous experiments. Both plus and minus races were found among the isolates from New Jersey, New York, Ohio, and Virginia. Minus races alone were found in Indiana and Connecticut, but only a few isolates from these states were obtained for experimental work. Both plus and minus races were found in one tree in New Jersey and in another in Ohio. Both races were found in close proximity in New York and New Jersey.

TABLE 1.—*Results of duplicate combinations of 14 monoascospore isolates of Ceratostomella ulmi*[a, b]

| | No. 5 | No. 8 | No. 10 | No. 11 | No. 12 | No. 13 | No. 14 |
|---|---|---|---|---|---|---|---|
| No. 1 | P | — | — | P | P | P | P |
| No. 2 | P | — | P | P | O | O | P |
| No. 3 | P | — | — | — | O | P | — |
| No. 4 | P | O | P | P | P | P | P |
| No. 6 | P | P | P[c] | P | O | — | P |
| No. 7 | P | — | P | — | O | P | P |
| No. 9 | P | P | P | — | P | P | P |

[a] P, perithecia; O, no perithecia; —, no cross made.

[b] No perithecia were formed when numbers 5, 8, 10, 11, 12, 13, and 14 were paired with one another nor when 1, 2, 3, 4, 6, 7 and 9 were paired with one another.

[c] Not. duplicated.

[3] Rawlins, P. E. Phytopathological and Botanical Research Methods. John Wiley & Sons, New York, 1933.

It has not been possible to differentiate races of the fungus morphologically or culturally except on the basis of their ability to form perithecia. Variants or sectors, strikingly different from the parent culture, frequently are produced by both monoconidial and monoascospore isolates. In 75 variants from one monoascospore isolate, the plus or minus characteristic either remained unchanged from that of the parent culture or the ability to form perithecia when crossed with certain test isolates was lost, the production of conidia, however, appeared to remain unchanged.—ROGER U. SWINGLE, Division of Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture, in cooperation with the Ohio Agricultural Experiment Station, Wooster, Ohio.

SAMUEL HENRY ESSARY

UNIV. OF MICH.